



ANIMAL HEALTH & WELFARE  
BIOSECURITY  
ENVIRONMENTAL PROTECTION  
SUSTAINABILITY OF ANIMAL HUSBANDRY

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# XIII INTERNATIONAL CONGRESS IN ANIMAL HYGIENE ISAH-2007

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June 17-21, 2007, Tartu, Estonia



ANIMAL HEALTH, ANIMAL WELFARE AND BIOSECURITY

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PROCEEDINGS VOLUME I

# XIII INTERNATIONAL CONGRESS IN ANIMAL HYGIENE

June 17–21, 2007, Tartu, Estonia



## “ANIMAL HEALTH, ANIMAL WELFARE AND BIOSECURITY”

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Editor: A. ALAND

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International Society for Animal Hygiene  
and  
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Tartu 2007

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# FOREWORD

On behalf of both the Organising Committee and the Scientific Committee, I am pleased to welcome you in Tartu, Estonia, to participate at the XIII International Congress of the International Society for Animal Hygiene (ISAH).

The ISAH ([www.isah-soc.org](http://www.isah-soc.org)) was founded in 1970 and has today members from 48 countries throughout the world. ISAH can be considered as a group of scientists contributing to efficient, sustainable animal farming with healthy animals, providing wholesome food in a sound environment.

Veterinarians and non-veterinary academic scientists (animal science, agricultural economics, engineers, microbiologists, public health professionals, epidemiologists etc., etc) and respective professionals in animal husbandry, who work and/or do research and education in the field of animal hygiene, can apply for a membership of ISAH, and are most welcome to attend ISAH congresses.

The first ISAH congress was held in Budapest in 1973. The last ISAH main congress took place in Warsaw, Poland in 2005 and the last in-between symposium in Saint-Malo, France in 2004.

Starting from Warsaw congress in 2005, the ISAH, considering the need for a more flexible and frequent exchange of scientific and practical knowledge, organizes its congresses every second year.

The present, XIII ISAH congress in Tartu, Estonia, in June 17–21, 2007 is organised under the device **"Animal health, animal welfare and biosecurity"**.

The scientific programme, trying to follow the scope of the ISAH and receive the feedback from modern animal husbandry and food production, concentrates with more profoundness on the following subjects: interaction between the environment and health and welfare of individual animal and herds; managing animal health in large dairy units; ensuring animal welfare during transportation and slaughter; economical implications considering animals' health; possibilities of precision livestock farming in maintaining good health and welfare of animals; measures for prevention the development and spread of diseases and pathogens in animals including those posing risk to human health (zoonoses); food safety relevant infections and contaminations such as residues in food derived from animals; influence of the animal production on the environment and public health.

The Proceedings from the XIII ISAH Congress are herewith presented. The papers on lectures from invited speakers, oral and poster presentations from 11 parallel sessions are included in this excellent compilation. In general, the printed contribution to the ISAH-2007 congress illustrates clearly the broad scientific field of the ISAH and related to it activities.

I hereby would like to express my most sincere gratitude in the address of ISAH-2007 organising and scientific committees. Special thanks go to Frens Conference Services for their excellent organizational and technical contribution and to AS Triip for their outstanding printing job of these proceedings. We also appreciate different companies and organisations for their considerable financial support which gave us the opportunity to keep the registration fees affordable.

Finally, we thank all participants, contributors, chairpersons, organisational and technical assistants for your considerable efforts – you made the ISAH-2007 in Tartu real success.

We wish you all interesting and pleasant congress and enjoyable stay in Tartu.

A. Aland  
Editor  
Chairman of the ISAH-2007 Organising Committee



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**Sunday, June 17, 2007**

**PLENARY LECTURE**



## HERD HEALTH MANAGEMENT AND QUALITY RISK CONTROL ON LARGE DAIRY FARMS

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### ABSTRACT

Large dairy farms commonly differ from small-holder or medium-sized farms in the sense that they need to be well-structured and organized, and should be considered as enterprises. The entrepreneur-like dairy farmers show other characteristics than average dairy farmers. They also have other demands regarding the veterinary services on their farm. The “sick cow approach” is no longer valid. If veterinary practitioners like to play a substantial role like consultant-coach on these large farms, they have to invest in new knowledge, skills and technologies which are currently not part of European curricula. Among the investment domains are herd health & production management (HHPM) services focussing on reducing costs and/or increasing income through operational management advice, farm economics, marketing & communication.

In addition, new programmes of quality risk control based on the HACCP (hazard analysis critical control points) concept and principles emerge as a consequence of new EU regulations like the General Food Law and the Hygiene Directives. In these programmes, quality comprises both the product (milk) and the production process, while animal health, animal welfare, and food safety/public health are exponents of such production process.

Both HHPM and HACCP are addressed in this paper. Moreover, as a practical link between the two programmes SWOT (strengths–weaknesses–opportunities–threats) assessment sheets and GDF (good dairy farming) guidelines are presented. It is concluded that HPM and HACCP can be rather easily integrated on the dairy farm, and that the veterinary practitioner is best positioned to play a pivotal role, provided that he/she is well prepared and willing to invest in the new skills and knowledge presented. Then, a sustainable, new veterinary market segment lays ahead.

### INTRODUCTION

Over the past decades we have seen a shift from small-holder mixed farms to more larger, mono-species, intensive dairy enterprises. New technologies-like for cattle housing, feed harvesting and feed mixing, milking – were introduced to meet the demands of increasing the milk production and labour productivity, necessary to cope with the smaller economic margins and to earn an income (Brand et al., 1996). At the same time it became clear that in order to manage large dairy enterprises, new skills and knowledge were paramount. Among these features are: [1] executing proper entrepreneurship, [2] set up an adequate organisation structure on the farm, including

assignment of responsibilities and tasks, and performance evaluation, [3] prioritising animal health and nutrition, and [4] design performance evaluation and risk analysis schemes.

The veterinary practitioner in this setting has to acquire new knowledge and skills too; the classical “sick cow” approach is no longer acceptable to the entrepreneur-like dairy farmer because diseases losses may be high and because the farmer is much more interested in disease prevention. The practitioner should turn into a consultant-coach for the entrepreneur-like dairy farmers to retain added value to these farmers (van Egmond et al., 2006). One way to perform this new task is to get experienced in herd health & production management services (HHPM) supporting the operational management. HHPM is founded on broad *clinical monitoring* of animals and their environment, searching for pending hazards and risk conditions, and evaluating herd performance data as well as the personnel.

A *SWOT assessment* is another crucial tool within HHPM. From available generic risk factor lists, a specific farm area like claw health or udder health is scored using area-specific SWOT sheets. At the end of such scoring, a *spider-gram* can be drawn showing the weak and the strong points of the farm. These results may trigger further action like interventions, further problem analysis or sampling for laboratory investigations.

Furthermore, because “quality” has become a leading issue in the EU, and hence in dairy farming, the practitioner should invest in skills and knowledge related to quality risk control. Quality must be considered in its broadest sense: from product safety (milk; meat) to animal health, animal welfare, public health. It has been stated earlier that the HACCP-like approach is the quality control concept best applicable to dairy farms as compared to ISO-9000-series and Good Dairy Farming guidelines, both for its farm-specificity and its merger with quality assurance systems further down in the food chain (Noordhuizen & Welpelo, 1996).

In this paper the forenamed programmes, services and concepts are addressed in a practical manner. Examples are given when appropriate. At the end it is concluded that veterinary practitioners could play a paramount role, once they are willing to invest for the future. When HHPM and HACCP are integrated, both farmers and practitioners can benefit.

### **Herd Health & Production Management, HHPM, services**

HHPM have been developed from single area (fertility) schemes to more holistic farm management approaches. However, still many farmers drop out because veterinarians tend to stick to their technical skills too much, instead of adding e.g. data or problem analysis protocols, biosecurity guidelines, or risk analysis schemes to their HHPM product.

HHPM must be **structured** and needs **planning** ahead; the ‘product’ must be **transparent** and clear to the farmer, it must be founded on **farmer’s demands** and priorities.

A *SWOT assessment* of the various farming areas will assist in determining the areas for improvement and prioritize them according to the farmer’s wishes. Through [www.vacqa-international.com](http://www.vacqa-international.com) you can get access to a website with, for example, such SWOT sheets for on-farm use. The SWOT assessment sheets function as follows (Cannas et al., 2006):

*Suppose you like to assess claw health problems. First of all, diagnoses have to be set (pictures provided in the website). Then the SWOT takes you along several clusters of items to be scored. Among these clusters are: Clinical Monitoring; Housing; Climate; Management; Other health disorders. The items within each cluster can be scored, commonly from 1–5 ranging from good–moderate–poor. Several of the items refer to risk conditions contributing to claw disorders, others refer to adjacent farming areas (e.g. lameness ~ oestrus expression ~ reproductive performance ~ feed intake ~ milk production). Scoring is conducted for a sample of*

cows in each of 4 lactation stages or as a group/herd average without lactation stage. At the end of the assessment the results are presented in a spider-gram and a histogram with colours from green (okay) to yellow (moderate; needs attention) to red (poor; immediate action required), while the items for improvement are listed for further interpretation and advice/intervention. Spider-grams can be used for evaluation of trends once a new assessment has been conducted both within farms as well as between farms. The data can be saved, and exported to PDF or printed.

Figure 1 shows an example of a screen of the VACQA-International website for the area of udder health

Currently SWOT assessment sheets are available in the areas claw health, udder health, herd fertility, milk production & nutrition, and calf rearing (4 periods). New SWOT sheets on Welfare & Cow Comfort, and on Public Health respectively are to be issued before the end of 2007.

The screenshot shows the VACQA-International website interface. The main window is titled 'Step 2 of 11 - Clinical Monitoring' and contains several assessment items with dropdown menus for 'Early lactation' and 'Mid lactation' stages. The items include:

- 2.1 Percentage of fresh cows with udder e... (Early: 1, Mid: 1)
- 2.2 Percentage of unbalanced and or blind... (Early: 3, Mid: 3)
- 2.3 Is hair clipped from the udders and th... season routinely? (Early: 1, Mid: 1)
- 2.4 Teat End Callosity Score, TEC score, re... (Early: 3, Mid: 2)
- 2.5 California Mastitis Test (CMT) testing results: (Early: 1)

A pop-up window titled 'Teat End Callosity Score, TEC score, results:' is overlaid on the right side. It contains the following text: 'This allows us to evaluate over-milking and milking machine function overall.' Below the text are two images of teat ends, labeled 'Score 1' and 'Score 2'. The 'Score 1' image shows a teat end with a smooth, rounded tip, while the 'Score 2' image shows a teat end with a more irregular, textured tip. The pop-up window also includes a search bar and a zoom level of 100%.

**Figure 1.** An example of the SWOT sheets from the VACQA-International website; the area of udder health monitoring

Once the hazards have been determined, the HHPM product contents can be designed. Broad **clinical monitoring** is the basis for each HHPM; it regards animals/herd, their environment and the management, and the data of the herd/farm. Monitoring is a rapid, cheap, and sufficiently reliable tool to track down deviations in performance, to assess potential hazards and risk factors,



to detect trends in performance, and to evaluate the effects of advice or intervention given earlier. Monitoring results trigger further action like problem analysis, expert consultation, risk analysis schemes, development of biosecurity assurance plans, design of specific working instructions, etc.

**Farm visits**, every 1, 2 or 4 weeks, depending on herd size, are pivotal in HHPM because the forenamed monitoring is conducted during such visits; the discussion with the farmer and farm workers about the results is crucial for proper understanding and follow up.

Farm visits have 3 components:

- [1] preparation while checking the latest events, the earlier advice given, and the state of herd performance;
- [2] the execution of the visit and the monitoring, including the interventions and the discussion with the farmer and farm workers;
- [3] the follow-up, comprising **problem analysis**, expert consultation, reporting. A written report of a farm visit is an essential element of HHPM. The same applies to the written reports regarding the problem analysis.

These 3 components are also the parts that need to be paid for in a commercial setting. Commonly only the [2] and [3] are the most relevant ones. Follow-up can comprise up to 2 to 3 hours after a farm visit.

**Preventive actions**, after routine monitoring and farm visits the third primary component of HHPM, are mainly focussed on tailor-made vaccination programmes, risk analysis schemes, biosecurity assurance plans, and, finally, quality risk management programmes. It must be stated here that investments in cattle welfare economically pays off. Adjustments contributing to optimising cow comfort (with the areas of housing; climate; feed & feeding; health; behaviour) result in less health disorders and better milk production (Noordhuizen & Lievaart, 2005).

### **Quality Risk Management programmes**

As stated above, “quality” in this context must be considered broadly. For the EU it has become a major drive to consumer protection (EC 178-2002; EC 852/853/854-2004). The European Commission has suggested to farmers to implement a HACCP-like programme to demonstrate the status of public health, animal health, animal welfare of their herd as well as of their products (milk; meat) to third parties (consumers; retailers; authorities). Earlier benchmarking also pointed to the HACCP concept as best applicable to dairy farms (Cullor, 1995; Noordhuizen & Welpelo, 1996) because of its simplicity, farm-specificity, low labour input and low documentation demands, low costs, and its basis in risk identification and risk management during the production process. The latter items have been named above under HHPM already.

Applying the HACCP-concept on dairy farms implies the following 7 principles (adapted after Cullor, 1997):

- A detailed description of the production process on the farm with all its steps in the format of flow charts and diagrams (which should be done anyway on large farms for organisational purposes!) by the HACCP-team which comprises the owner, the manager, the chief veterinarian, the nutritionist, the farm-economist.
- Identification of major hazards (diseases) and their associated risk factors in the areas of animal health, animal welfare and public health/food safety. (This is usually done in a more qualitative and generic way during HHPM services, but must be done here in a much more formal and structured way!)

- Definition of critical control points (CCP) and points of particular attention (POPA) to control the risks of concern throughout the production process. (POPAs fail to meet all the formal criteria as set for CCPs but still are considered relevant for risk reduction)
- The setting of standards and their tolerance level (physical entities) and targets (for biological entities) around each CCP and POPA.
- Design of a monitoring system involving CCPs and POPAs, frequency of monitoring, method of monitoring, the related record, and person responsible for it (this item too is somehow addressed in HHPM but again is formalised here).
- Definition of corrective and preventive measures at each CCP and POPA (is commonly addressed in HHPM once a [pending] problem has been detected).
- Verification of the proper functioning of the HACCP-like plan through internal reviews and screenings, and by external audits; the provision of necessary records.

These 7 principles are to be translated into the 12 steps of developing a tailor-made hence farm-specific HACCP-like plan. These 12 steps are addressed during the conference workshop to show its feasibility and practicality. Parts and examples from the handbook on a HACCP-like plan will be shown also. Fig. 2 on the next page shows an outline of a dairy farm production process flow chart; Fig. 3 shows a part of the HACCP-like handbook.

A crucial element in the **HACCP**-like approach is the fact that we **need to structure and formalise** what we –maybe- have not yet done so far in HHPM.

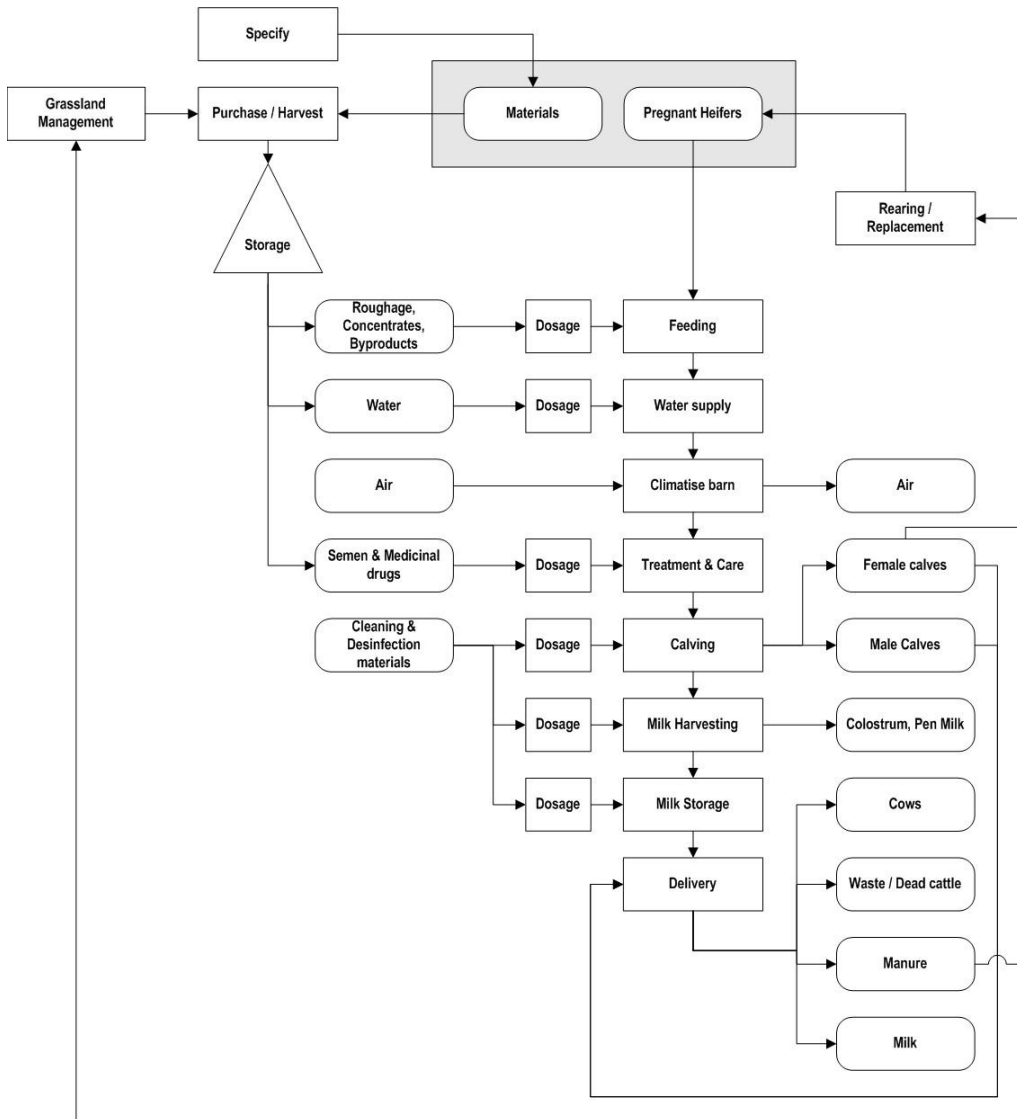
In order to determine whether an identified risk on a dairy farm is an actual risk or not, we can follow one of 3 possible routes:

- conduct a qualitative risk weighing in the HACCP-team on the basis of its probability of occurrence X expected impact ( $R = P * I$ ) (Lievaart et al., 2005)
- apply methods of adaptive conjoint analysis (from marketing sciences to assess experts' opinions on a certain issue) yielding a ranking of risk factors in a semi-quantitative way (van Schaik et al., 1998)
- apply formal, quantitative observational-analytic epidemiological studies yielding odds ratios or relative risks (Noordhuizen et al., 2000).

A CCP can only be defined as such if formal criteria are met; these are that the CCP is associated with the hazard of concern; that it is measurable or observable; that standards/tolerances are known; that corrective measures are available; that these corrective measures restore full control of the process again after a breakdown. POPAs usually are lacking the third and the last criterion; the main reason is that animals are biological, not physical, entities, and that, hence, biological variation exists (e.g. sero-titres distributions).

In animal husbandry, standards and tolerances known for physical processes are not very common. An example however is the temperature of the water for cleaning the milking machine ( $80^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ) which is a true CCP. Therefore, in dairy farms we will have much more POPAs, which at least can be supportive to reduce the risk. Moreover, items like breed

or houses can be risk factors for e.g. lameness, but a farmer will not accept our “advice” to replace them.



**Figure 2.** Example of an overall dairy farm production process diagram (flow chart)

Item no.	CCP/ POPA	Standard Tolerance	Monitoring			Instruction (prevention)	Corrective measures	Records
			How	Freq.	Who			
T <sub>1</sub>	POPA	Use only proper drug	Check label	At use	Farmer	“Use of drugs”	Use proper drugs Evaluate other drugs Consult the vet	“Drug Record” (R <sub>t</sub> )
T <sub>2</sub>	POPA	No residues	Check drugs (R)	At delivery	Farmer	“Use of drugs” “Drug delivery”	Respect the with- drawal periods	(R <sub>t</sub> )
T <sub>3</sub>	POPA	Dosage in DAP.	Check syringe	At use	Farmer	“Use of drugs”	Adjust dosage	(R <sub>t</sub> )
T <sub>4</sub>	CCP	Cow ID No tolerance	Visual	At drug use	Farmer	“Use of drugs”	Mark the cow	(R <sub>t</sub> )

T= cow treatment step in the process; POPA= point of particular attention; CCP= critical control point; DAP= drug advisory plan of the veterinarian for the herd. R = records. R<sub>t</sub> = Records regarding treatments

**Figure 3.** Example of a part from the HACCP-like handbook, regarding the process component of cow treatment (T<sub>1, 2, 3, 4</sub> refer to items in the handbook)

An on-site monitoring scheme involves the CCPs and the POPAs. Its function is fully comparable to that used in HHPM, but under HACCP it is –again- much more structured and formalised.

The HACCP records, needed to prove to third parties (authorities; retailers; consumers) that the quality risk management plan is in place and adequately functioning, comprise components like a Daily Events & Calamity Log; Intervention Sheets; Herd Treatment Advisory Plan e.g. for mastitis; a Good Medicine Application code of practice; Performance Records; Quality Control Sheet; Laboratory Examination Sheets.

Several of these records will already show up in a properly designed and functioning HHPM service. They are also available at [www.vacqa-international.com](http://www.vacqa-international.com).

This website comprises many templates of a HACCP-like handbook (about 100 pages) and provides examples of hazards & risks lists; flow charts and diagrams; CCP & POPA lists; monitoring schemes; intervention schemes. These templates can be used for adaptation to the regional and local (farm) situation. Risk factor lists are generic examples.

It must be stated that the application of quality risk management according to the HACCP concept would be senseless if not the proper attitude and mentality has first been adopted by both the farmer/owner and the veterinarian as well as the farm workers. A way to properly deal with such adoption refers to the marketing of “protocols” of Good Dairy Farming codes of practice (GDF) and working instructions associated with these protocols. GDF codes are guidelines and address different farming areas like Hygiene, Feed Harvesting, Feeding Management, Milk harvesting, Colostrum Management.

GDF guidelines largely encompass the more generic types of risk factors which are hence not specific for a certain disorder. The veterinary practitioner is well-positioned to design such guidelines, market them and start training and coaching in implementing these on the farm with the manager and the farm workers. When these type of working instruction are adopted on the farm, the foundation is built to expand to HACCP-like applications.

On the [www.vacqa-international.com](http://www.vacqa-international.com) website different examples of such GDF guidelines can be found too. They can be adapted to the particularities of the local (farm) situation. By the end of 2007 a book on the various applications of HACCP with many field examples will be issued by Wageningen Academic Publishers.

## DISCUSSION AND CONCLUSIONS

Applying HHPM to large dairy farms means more than involving veterinary technology alone. If we like to keep these enterprises as our client, we have to enter other domains (van Egmond et al., 2006).

The first domain is that of the farm organisation: How is it set up as a business? Are there different farm units being distinguished for better management? And if so, are tasks and responsibilities defined for farm workers? How is performance being evaluated? etc

Next domain is the marketing and business administration. We need to be able to follow the entrepreneur in his ways of thinking. A particular element of this domain is communication in its broadest sense (oral and written communication; raising the proper questions; adequate listening; conflict handling; investing in contact moments; moderating discussions; properly convincing people).

The third domain is –next to adequate veterinary-zotechnical knowledge and skills – animal health economics. A veterinary coach-consultant must be able to deal with disease loss estimation and cost-benefit assessments of advisory programmes or interventions. A specific area in this domain is “behavioural economics”, the irrationality in decision-making processes based on issues like perceptions, emotions, vision on the outside world, social standing, pleasure in his enterprise. Again, the practitioner should be able to follow the entrepreneur and recognise the signs of such behaviour in order to discuss at the same “wave length” as the farmer/manager.

If the practitioner detects during a self-evaluation session several blanc spots in his professional profile, related to the forenamed domains, he better invest first in acquiring such knowledge or skills before jumping too quickly and too deep into HACCP-like applications. A client is lost more rapidly than won. Different courses on these subjects are given, most probably by branches other than veterinary.....

From the preceding paragraphs it is clear that HHPM and HHCP-like applications can be easily integrated. They both have the same client, the farmer; the scope of quality risk management through HACCP is wider and more at the tactical/strategic level, while HHPM is at the operational management level. HACCP is also much more structured and formalised than HHPM. When conducting HHPM and/or HACCP it must be absolutely clear to everyone that clinical intervention activities (like claw trimming; calf dehorning; treating endometritis) must be strictly separated from coaching-consulting activities. The farmer should not be confused and his concentration must be focussed on the work under hand; on the other hand, the practitioner should not loose too much time being distracted by such clinical work while coaching/consulting. For the same reason, the veterinarian should leave his mobile phone in his car, not being tempted to denigrate the farmer!

The main reason for integrating HHPM and HACCP – for example in a developmental pathway – is that quality control on dairy farms must be conducted in a “bottom up” sense instead of a top-down approach. The latter will never lead to full adoption by the farmer unless being forced by e.g. authorities or retailers. By integrating the two, the benefits for the farmer are much bigger.

SWOT assessment tools and Good Dairy Farming codes of practice as proposed here are the practical links between the two types of services.

The contemporary veterinary practitioner can play a paramount role in these new services if he/she is willing to invest in new knowledge, technology and skills first. Then, a new large market segment lays ahead, while his/her pleasure in new activities will increase.

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**Monday, June 18, 2007**

**PLENARY LECTURES**





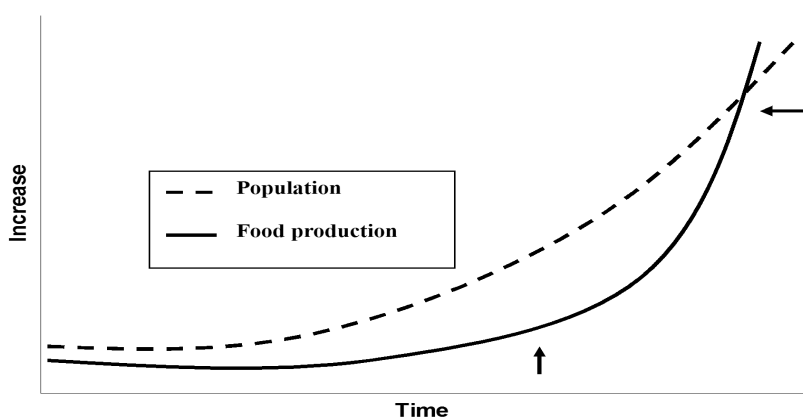
## THE GROWING ROLE OF ANIMAL HYGIENE FOR PRODUCING FOOD OF ANIMAL ORIGIN

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### THE CHANGES

It is striking that the changes in food production (especially in the production of food of animal origin) in the last 15 years have by far exceeded the changes that took place in the period of time from the turn of the 19<sup>th</sup> until 1990. It has been mostly speculated that the reason for that is the endless sequence of real and perceived food crises such as the BSE episode, the sudden Salmonella Enteritidis emergence, and many other so called food scandals like dioxin in feed and food, resistance in bacterial pathogens and so on. I dare to argue that the underlying “problem” of the changed attitude of consumers and the society towards food is a very positive one: in many parts of the world, food production exceeded the demand for food. Figure 1 shows a very simplified graph demonstrating the effects of the relationship between population growth and food production. The solid line in Figure 1 symbolizes the population growth over time; the dotted line symbolizes the growth of food production. Due to a more rapid growth of the population than the growth of the food production, there has been until recently always a need for more food – wars and a rapid urbanisation (fewer farmers have to produce food for more urban people) have even widened the gap between the two lines until we decided to purposefully intensify food production (first arrow). As the Figure 1 shows (second arrow), it has been only for a very short period of time that food is being produced in a way that almost everybody (unfortunately only in the developed countries) has access to an abundant food supply.



**Figure 1.** The theoretical relationship between population growth and the increase of food production

In contrast to many a modern “consumerist” I think that intensifying food production has been one of the great achievements of mankind – although it has to be admitted that, as so often, this achievement has its price: **we have to learn how to produce food in a way that supplies everybody with abundant, wholesome, nutritious and safe food AND to simultaneously maintain our resources, protect our environment and keep animals for food production under conditions that allow them a decent life fulfilling their needs for animal well being** – in other words, the task is: building up an efficient and yet sustainable animal production for a socially acceptable food supply feeding the world.

These changes have both an impact on the market that are mainly due to the growing free global trade with feed, animals, and raw material for food and food itself, and an impact on the legal framework that are responding to the growing demands of the society.

**The market** has started to look not any more only for the lowest price, but also for superior quality, traceability, guarantees for safe products and for ethical values such as “environmentally sound”, “sustainable production”, and “animal friendly”.

**The legal framework** for the new conditions under which food production is expected to take place has started to change with the new definition of the principles for food safety by the Codex Alimentarius in 2000. Accordingly, the rules of the World Organization for Animal Health (O.I.E. – since recently also responsible for food safety and animal welfare), the rules of the World Trade Organization (WTO) and of the Agreement on Sanitary and Phytosanitary Measures (SPS) reflect the new expectations as well as the legal framework for feed and food safety in the now 27 EU-member states, which is summarised in the Reg. (EC) 178/2002.

The major new principles of the paradigm shift that is reflected in this new regulatory framework are:

- strengthening of the responsibility of the producers for safe products and state of the art production procedures
- process optimisation rather than end product inspections
- risk-oriented safety procedures (e.g. HACCP) and risk-oriented controls and inspections
- setting targets rather than prescribing every detailed procedure
- the “third eye principle” (auditing and certification)
- public-private partnerships (self controls, neutral controls and governmental control of the control)
- the primary production (feed production, animal husbandry) has to be part of the food safety system along the food chain.

## THE CONSEQUENCES

These changes have, of course, quite drastic consequences, which mainly put pressure on the farming community that has never been exposed to such a speed of change in the past. It is by far not any more sufficient to produce “as much as possible at as low as possible costs”. Agricultural production becomes more and more market-oriented. The probably most drastic change for the farmers is the fact that, with abundant food available and with the possibility for food processors and retailers to buy any raw material and any food from everywhere, the need of national self-sufficiency is gradually decreasing. In other words: affluent societies that buy food from all over the world lose their willingness to pay farmers for overproduction and/or for products that do not meet the demands of the market. There is also a decreasing willingness of the government to “fix

all the problems” that farmers may run into (contagious animal diseases, improper production processes leading to economic losses and suboptimal products).

To maintain a sound livestock production as basis for a competitive production and supply of food of animal origin, the following major tasks must be fulfilled:

1. eradication of contagious (notifiable) diseases and protection of the national livestock against the introduction of foreign and emerging diseases;
2. controlling and minimising the multitude of endemic (multi-factorial) diseases impairing animal performance and animal welfare;
3. controlling, minimising or eradicating zoonotic pathogens and chemical and physical risks to human health at herd level (pre-harvest food safety);
4. optimising the husbandry and animal care conditions to ensure animal well being. (including transport and stunning before slaughter);
5. protecting the environment against adverse effects stemming from animal husbandry (emissions, improper waste management, ground water pollution);
6. assuring the compliance with the necessary measures to be taken (internal and external audits, certification and traceability) to tackle all these tasks.

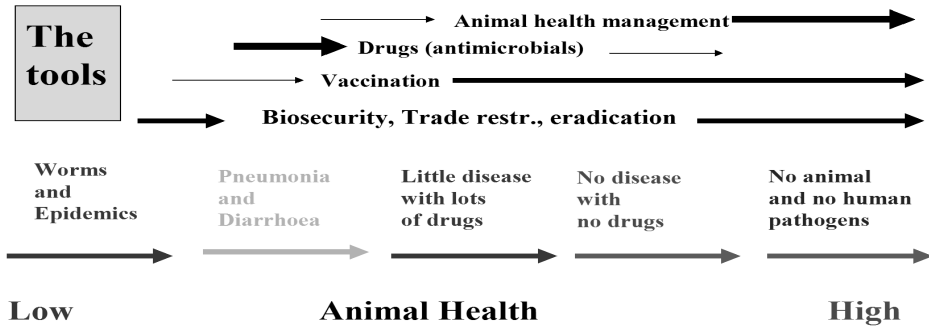
### ANIMAL HYGIENE’S CONTRIBUTION TO ANIMAL HEALTH

“Animal Hygiene” is the discipline of veterinary medicine that is not focussing at animal disease, but on animal health. In the last decades, especially in the framework of the International Society for Animal Hygiene (ISAH), the scope of “animal hygiene” has been broadened from “just” animal disease prevention to:

- **animal health** in the widest possible sense (freedom from disease, freedom from suffering and pain, freedom from pathogens harmful to animals and humans);
- **food safety** at herd level (no microbiological, chemical or physical contamination of meat, milk and eggs, and minimisation of bacterial resistance);
- **environmental protection** in all areas that are affected by animal production (waste management, protection of soil and ground water and minimisation of emissions from animal husbandry).

Figure 2 shows a graph that tries to illustrate that animal health is not a “Yes” or “No” issue, but a quantitative criterion that can be classified as “Low” or “High”, which makes it possible to define improvements.

**Animal Health is**  
**...not a simple „No“ or „Yes“, but a complex „Low“ or „High“**



**Figure 2.** Improvements of animal health over time and the major tools of the animal health management

In the light of this definition, animal hygiene is involved in all “new” challenges that the farming community is increasingly facing.

### 1. Biosecurity

Apart from complying with the international regulations for the trade with animals and animal products, and the national regulations on the protection of the national livestock (monitoring, surveillance and early warning systems), a set of precautionary measure have to be taken at farm level. First of all, there is a need to maintain at all times the awareness of farmers and veterinarians, that any symptom of disease might be a symptom of a contagious (notifiable) disease. It must be stressed that the “first line of defence” against the tread of outbreaks of contagious diseases is the farmer and the field veterinarian, not the state veterinarian, who can only take actions if somebody indicates a suspicion of disease. It has been experienced in the last decades that statistical sampling for monitoring for antibodies and/or causative agents of contagious diseases are rather providing a false sense of security – the targeted diagnostic clarification of any clinically suspicious case is in all events more likely to early detect an outbreak. If such targeted diagnostic measures are part of the daily considerations of the farmer and the consulting veterinarian, a major condition of animal hygiene, i.e. preventive veterinary medicine is fulfilled.

Additionally, of course, animal hygiene is teaching the basic rules of biosecurity such as shower-in, changing of boots and overalls, restriction of visitors, quarantine and isolation measures for animal replacements etc.

### 2. Endemic diseases

If epidemic diseases are eradicated and “kept out” of our herds and flocks, the impact of animal specific pathogens (that often only together with adverse factors develop disease) must be minimised. GAP (Good Agricultural Practices – good stockmanship and good husbandry) and GVP (Good Veterinary Practices) need to be implemented. Only if the animal and people flow is

oriented towards a constant reduction of infection chains within the herd or flock and along the animal production chain from breeding up to slaughter, and only if the living conditions of the animals (ventilation, cleaning and disinfection, care and proper feeding and watering) are constantly being optimised, there is the chance to maintain and even improve the health of the animals. Appropriate vaccinations of the animals and the prudent use of antibiotics (no prophylactic use, and in case of disease: as much as necessary and as little as possible) are major components of GVP.

### **3. Pre-harvest food safety**

After having brought the “classical” food safety risks (tuberculosis, brucellosis, trichinellosis) under control, more and more “new” food safety risks emerge that cannot be controlled by the traditional meat inspection, which depends on inspecting the carcass. Risks such as Salmonella, Campylobacter, Listeria, residues and toxins, do not result in clinical disease (which could be detected in the flock or herds), and they do not cause any pathological-anatomical lesion (which could be detected at the slaughter line). And, even if they could be detected at the slaughter line (e.g. bacteriology or any other analytical test), they cannot be removed from the carcass. The only way to deal with these risks to human health is to prevent their entering the flock or herds of animals. Observing the basic hygienic requirements (shower-in, change of boots and overalls, rodent and pest control, cleaning and disinfection between production cycles, vaccinating where appropriate, compliance with withdrawal times and prudent use of antibiotics) are the major preventive measures for reducing the at-herd-level food safety risks. Additional monitoring and surveillance systems to identify higher risk flocks herds and to implement measures in these high-risk flocks and herds for mitigating the risks in question, will contribute further to reasonable and effective pre-harvest food safety programmes.

### **4. Good Agricultural Practices (GAP)**

Apart from disease prevention, the animals deserve a decent life and any prevention from suffering and pain. The animals physiological needs need to be met and the species-specific behaviours need to be taken into consideration to guarantee. It goes without saying that the husbandry system is having the highest impact on the degree of the “animal friendliness” of the rearing conditions. However, whereas in recent decades the husbandry system has been almost exclusively made responsible for the well being of food animals (e.g. poultry batteries vs. free-range, sow crates vs. sow group housing etc.), it has become increasingly obvious that the intensity and quality of the animal care (the degree of stockmanship) can even override the positive or negative effects of certain husbandry systems. Optimal feeding, water supply, veterinary care and intensive observation of the animals and taking care of their needs are at least as important as the husbandry system.

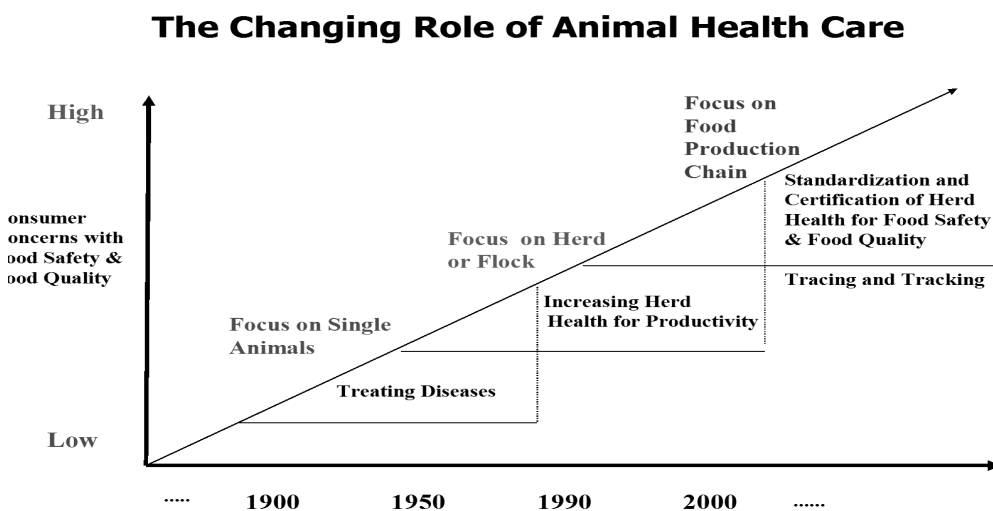
### **5. Environmental protection and waste management**

Sustainable production methods for food of animal origin ask for minimisation of adverse emissions from any livestock production facility and for a responsible waste management. Appropriate feeding strategies (e.g. phytase supplementation), reducing emissions from waste storages (e.g. covering of slurry tanks), and emission reduction by using filtering systems (e.g. biofilters), are as important as a responsible use of veterinary drugs and disinfectants that potentially contaminate animal wastes.

## 6. Traceability

Any market-oriented food production is only competitive and providing trust, if the production procedures are completely transparent. The major precondition for transparency is a seamless system for tracking and tracing back and forth along the production chain from “plough to plate”. Modern identification, data recording and information systems provide more and more possibilities for a transparent production flow. Third party auditing and certification procedures will “produce” as much trust for consumers and markets as the guarantee that recall actions are possible in case of any failure in the production chain. Fully integrated, corporate production systems such as certain poultry, egg, milk and pork production chains have already started to implement their own data recording, tracing and information system. However, first independent tracing and tracking systems provided by third party companies start to be offered in the market such as the software system “ScoringAg” (Scoring System, Inc., USA), which can be used by groups of producers that have a steady product and information flow without being fully integrated in corporate terms.

Figure 3 shows the theoretical change of the animal care management systems over time from curing single diseased animals to the sustainable production of high quality food.



**Figure 3.** The changing animal care systems over time

Implementing all components of “Animal Hygiene” as integral parts of Good Agricultural Practices and Good Veterinary Practices into the daily production procedures in livestock and into the veterinary service, the production of food of animal origin will continue to change from single animal care actions for curing diseased animals by therapeutic efforts to flock and heard health improvements for maximising the economic output of the livestock operations on to a **transparent and socially acceptable production of wholesome, healthy and safe food produced under sustainable production conditions.**

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## **ANIMAL HEALTH, ANIMAL WELFARE, BIOSECURITY AND ENVIRONMENTAL PROTECTION AS MAJOR COMPONENTS OF SUSTAINABLE ANIMAL PRODUCTION**

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### **SUMMARY**

European consumers are becoming increasingly concerned with safety and animal welfare in food production. They want to know more about breeding methods, fattening procedures and animal husbandry, prevention and eradication of severe animal diseases, meat inspection, and recently also about the sustainability of animal production in general. EU member states are obliged to improve the health status of farm animals, to reduce the human health risk from the consumption of food of animal origin and to guarantee free intra-Community trade. When Austria joined the EU on 1 January 1995, this obligation led to a considerable extension of the scope of tasks to be performed by the veterinary service and to the implementation of new methods in food production and monitoring.

#### **Animal health**

The EU has put in place a large number of directives, decisions and regulations to ensure and monitor the health status of farm animals and has established common rules for the control of epidemic and endemic diseases as well as additional guarantees and surveillance programmes. Food animal production systems vary in different regions of the world. In countries practising intensive animal production, thousands of animals are kept in confinement in large operations. In other countries, like Austria, production is less intensive (with the exception of poultry production), largely pasture based and often small in scale. All EU member states have established veterinary service systems for implementing “herd health programmes” to improve animal health based on process optimisation. Farmers are at the beginning of the food production chain and therefore have a great responsibility in safeguarding animal health and the quality of animal products. Improvement of animal health is also a great challenge for veterinarians. In Austria, we set up the “Animal Health Service” (AHS) in the 1990s, with farmers and veterinarians working together to increase the productivity of the farms, to improve the quality of food of animal origin and to establish a quality assurance system. The AHS, in cooperation with the Austrian Veterinarian Association, has defined specific rules of cooperation between veterinarians and farmers. Regular veterinary audits are undertaken by private veterinary surgeons who are members of the Animal Health Service.

#### **Animal welfare**

The EU has already taken various practical steps to secure real improvements in animal welfare in order to respect the basic five freedoms: freedom from discomfort, from hunger and thirst, from fear and distress, from pain, injury and disease, and freedom to express natural behaviour. The



European Union has already put in place welfare standards for animals kept for farming purposes, such as laying hens, calves and pigs. The Community Action Plan (CAP) on the protection and welfare of animals, adopted in January 2006, also responds to the principles of the Amsterdam Treaty. Recent CAP reform measures have introduced the principle of cross-compliance with various standards for beneficiaries of direct payments, including animal welfare standards, from 2007. The “Integration of animal welfare in the food quality chain: from public concern to improved welfare and transparent quality” is of great importance. In January 2007, a new regulation on the welfare of animals during transport came into force. Banning of long distance transport of live animals for slaughter or further fattening would be a sensible step because animals would not be subjected to long periods in transit. The protection of animals from avoidable suffering, pain or damage during transport to the slaughter facility as well as the topics of stunning and exsanguination is currently at the centre of hot public debate.

### **Biosecurity**

Biosecurity is the prevention of disease-causing agents entering or leaving any place where farm animals are present. Biosecurity measures are of special importance in the case of an outbreak of an exotic notifiable disease such as Foot and Mouth Disease (FMD), Classical Swine Fever (CSF), Avian Influenza or infections with Bluetongue virus in ruminants. The main risk factors responsible for spreading disease include farm-to-farm movement of infected livestock, contact with animals and their excrements, clothes, boots, vehicles and equipment. Disease can also be spread by other means, such as wildlife, other vectors or airborne transmission. Implementing biosecurity measures as Standard Operating Procedures (SOPs) helps ensure that those people working with farm animals or coming into contact with them do not spread disease.

### **Environmental protection**

Protection of the environment requires activity on many different fronts – from limiting global environmental threats (such as global warming, or greenhouse gases), to safeguarding individuals from the effects of poor air quality or toxic chemicals. Actions to protect the environment also provide benefits in improved energy efficiency and more efficient use of resources, such as re-use, recycling and recovery of waste. From the veterinary point of view, the safe utilisation of animal by-products (i.e., products of animal origin that are not intended for human consumption) such as animal carcasses, catering waste, butcher and slaughterhouse waste, blood, pet animals etc. is of special importance. The collection and decontamination of fallen stock in rendering plants according to Regulation 1774/2002/EU is also of great significance in terms of biosecurity. Animal by-products can be processed not only in conventional rendering plants but also in a wide range of other technical and biological processes, as for example in approved biogas or composting plants throughout the EU.

### **Sustainable development**

A widely used international definition of sustainable development is: “*development which meets the needs of the present without compromising the ability of future generations to meet their own needs*”. At the beginning of the 21<sup>st</sup> century the public is becoming more and more aware that the current model of development is unsustainable and that our way of life is placing an increasing burden on the planet. Global energy demand could double as a result of population growth in the next fifty years, global water use has more than tripled since 1950, and production, distribution

and consumption of food is responsible for approximately 25% of total greenhouse gas emissions. It is in our long-term best interests as veterinarians to play an active part in contributing to a more sustainable development in the production of food of animal origin. So let's make a start in this direction in our everyday work "along the food chain" and thus pave the way towards sustainable animal production.

## THE “HYGIENE PACKAGE” – A NEW APPROACH TO FOOD SAFETY

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### BACKGROUND

Following several disease outbreaks and food contamination scandals in Europe in recent years, the Commission adopted the White Paper on Food Safety in 2000. This White Paper contains a number of recommendations aimed at increasing food safety, improve the traceability of food products and regain consumer confidence in the food industry. To this end a package of proposals for new legislation on food and feed has been prepared with the following characteristics: responsibility for food safety lies with the establishment operator, while the competent authority of the Member State verifies correct implementation of the new rules. Production should be based on good hygienic practice and HACCP principles and products are subject to microbiological criteria and temperature limits. The proposals deal with a variety of food types and cover the entire food chain (“from stable to table”).

### General Food Law

The general food law (Regulation (EC) No. 178/2002<sup>1</sup>) lays down guiding principles and establishes common definitions. Furthermore, the Regulation puts the overall responsibility for producing safe food on the food business operator. It requires the food business operator to have a system in place enabling them to identify the immediate supplier(s) and immediate customer(s) of their products in order to ensure traceability. Other issues that are covered within this Regulation are the principles of risk analysis, the precautionary principle and withdrawal of food from the market by the food business operator if safety is at stake. Finally, it lays down the principles and requirements for the rapid alert system for food and feed and for the establishment of a European Food Safety Authority.

### Hygiene package

The “hygiene package” consists of a total of five legislative parts, of which four were adopted in April 2004, and provided the Member States and the stakeholders with a preparatory period of 18 months before becoming applicable with effect from 1 January 2006. The package consists of the following parts:

- Regulation (EC) No 852/2004 on the hygiene of foodstuffs<sup>2</sup>
- Regulation (EC) No 853/2004 laying down specific hygiene rules for food of animal origin<sup>3</sup>

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<sup>1</sup> OJ L 31, 1.2.2002, p. 1

<sup>2</sup> OJ L 226, 25.6.2004, p. 3

<sup>3</sup> OJ L 226, 25.6.2004, p. 22

- Regulation (EC) No 854/2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption<sup>4</sup>
- Council Directive 2002/99/EC<sup>5</sup>, which deals with animal health issues
- Directive 2004/41/EC<sup>6</sup>, which repeals the old legislation, a total of 17 Directives.

#### **Regulation (EC) No 852/2004**

The Regulation lays down general hygiene requirements to be respected by food businesses at all stages of the food chain including primary production. The Regulation does not apply to small quantities of primary products supplied directly by the producer to the final consumer or to local retail establishments directly supplying the final consumer.

The Regulation requires all food business operators to put in place, implement and maintain a permanent procedure based on Hazard Analysis and Critical Control Point (HACCP) principles with the exception of those involved in primary production. Food hygiene is the result of the implementation by food businesses of prerequisite requirements (such as concerning infrastructure and equipment, pest control, water quality, personal hygiene, etc.) and procedures based on the HACCP principles. The prerequisite requirements provide the foundation for effective HACCP implementation and should be in place before a HACCP based procedure is established. The prerequisite requirements to be respected are laid down in an annex of the Regulation. The Regulation allows the HACCP based procedures to be implemented with flexibility so as to ensure that they can be applied in all situations. Guides to good practice for hygiene and for the application of the HACCP principles developed by the food business sectors themselves, either at national or at Community level, should help businesses to implement HACCP-based procedures tailored to the characteristics of their production.

In addition, the Regulation requires food businesses to be registered with the competent authority, this being a simple procedure whereby the competent authority is informed about the address of the establishment and the activity carried out.

#### **Regulation (EC) No 853/2004**

The Regulation lays down the hygiene requirements to be respected by food businesses handling food of animal origin such as meat, live bivalve molluscs, fishery products, raw milk and dairy products, eggs and egg products, frogs' legs and snails, collagen and gelatine at all stages of the food chain. The Regulation does not apply to retail, which for food hygiene purposes means all activities involving direct sale or supply of food of animal origin to the final consumer. In such cases Regulation (EC) 852/2004 will apply. Establishments (except those carrying out only primary production, transport operations, the storage of products not requiring temperature controlled storage conditions or most retail operations) handling products for which the Regulation lays down requirements in an annex, must be approved. Approval procedures involve an on-site visit by the competent authority to verify if the establishment fulfils all the requirements concerning infrastructure, equipment and hygiene.

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<sup>4</sup> OJ L 226, 25.6.2004, p. 83

<sup>5</sup> OJ L 18, 23.1.2003, p. 11

<sup>6</sup> OJ L 195, 2.6.2004, p. 12

**Regulation (EC) No 854/2004**

The Regulation deals, among other things, with the official controls of animals sent for slaughter, official controls with regard to fresh meat, fishery products, raw milk and dairy products and with procedures concerning imports. Modern meat inspection should be based on risk assessment and should prevent cross contamination in the slaughter hall. In addition, meat inspection can be improved by imposing stricter hygiene measures at the farm level and by requiring the farm operator to send relevant management and health information to the slaughterhouse for those animals that are to be slaughtered in the next 24 hours, called food chain information. These principles have been introduced in the Regulation.

**Regulation (EC) No 882/2004 (Official feed and food controls)<sup>7</sup>**

The Regulation on Official Feed and Food Controls is the result of a review of the existing Community rules on the subject, which were adopted separately for the animal feed sector, the food sector and the veterinary sector. It covers the entire range of activities covered by feed and food law, including animal health and animal welfare. It applies with effect from 1 January 2006, except for the provision on financing of official controls which applies with effect from 1 January 2007.

As a consequence of the new rules, the Member States have to reorganise their official controls systems so as to integrate controls at all stages of production and in all the concerned sectors, using the ‘farm to fork’ principles. They have to submit and annually update a general control plan for the implementation of feed and food legislation and to report annually on the implementation of that plan. National control plans and reports shall take into account guidelines drawn up by the Commission (as mentioned under point 9).

There will also be an evolution of the Community approach to controls. At present, Community controls in the Member States and in third countries are organised largely on a sectoral basis and are related to the mandates the Commission has in different sectoral legislation. By means of this Regulation the Community approach to controls will evolve. The Food and Veterinary Office’s role will be essentially based on audit with the main purpose to verify the efficiency of the control systems in the Member States and auditing the compliance or equivalence of third country legislation and control systems with EU rules. The requirement for all Member States to submit a multi-annual control plan will facilitate the carrying out of these audits. Account will also be taken of Member States’ own audits and of their annual reports.

The Regulation provides for a set of general rules applicable to the official controls of all feed and food at any stage of production, processing and distribution, whether produced within the EU, exported to or imported from third countries. In addition to these rules, there are other specific control measures which are important in order to maintain a high level of protection and therefore must be kept in place. This is, for example, the case for the specific veterinary control rules on imports of animals and food of animal origin or for the specific controls rules for organic products.

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<sup>7</sup> OJ L 191, 28.5.2004, p. 1

## Implementing measures

### 8.1. Implementing measures

A wide range of implementing measures has been adopted on the basis of the hygiene package as foreseen in Article 12 of Regulation (EC) 852/2004, Articles 9 and 11 of Regulation (EC) 853/2004 and Articles 16 and 18 of Regulation (EC) 854/2004.

The measures laid down in Commission Regulation (EC) 2074/2005<sup>8</sup> include provisions concerning food chain information, fishery products, recognised testing methods for detecting marine biotoxins, calcium content of mechanically separated meat, lists of establishments, model health certificates for a number of products (frogs' legs, snails, gelatine and collagen), a derogation for foods with traditional characteristics and a number of amendments to Regulations (EC) No 853/2004 and (EC) No 854/2004.

### 8.2. Transitional arrangements

The principle of granting transitional arrangements was agreed by the European Parliament and the Council through Article 12 of Regulation (EC) No 852/2004, Article 9 of Regulation (EC) No 853/2004 and Article 16 of Regulation (EC) No 854/2004. Transitional arrangements in respect of certain new provisions have been taken to permit a smooth change-over from the old to the new regime.

The measures laid down in Commission Regulation (EC) 2076/2005<sup>9</sup> include provisions concerning stocks of food of animal origin, placing of food of animal origin on national markets, materials bearing pre-printed health or identification marks, marking equipment, health import conditions, food chain information, composition criteria for minced meat, use of clean water, raw milk and dairy products, eggs and egg products, training of slaughterhouse staff, certification of establishments, accreditation of laboratories carrying out official controls and some amendments to Regulations (EC) No 853/2004 and (EC) No 854/2004.

### 8.3. Examination of meat for *Trichinella* parasites

The adoption of Directive 2004/41/EC on 21 April 2004 by the European Parliament and the Council resulted in the repeal of Council Directive 77/96/EEC, which specified in detail the examination for *Trichinella* of carcasses of swine, horses and other susceptible species. Commission Regulation (EC) 2075/2005<sup>10</sup> has retained many elements from the previous legislation such as the sampling procedure, the various examination techniques in the laboratory and the derogations granted. However, at the same time the Commission Regulation has introduced a number of new elements to increase food safety for the consumer and facilitate the sampling procedure for those establishments where the parasite has not been encountered for a long time. The new elements are the following:

- A larger amount of sample has to be collected and examined from those animal species that pose the greatest risk for infecting humans, mainly horses and wild boar;
- Freezing is no longer allowed to replace the examination of horsemeat (because in this host certain *Trichinella* species such as *T. spiralis*, *T. pseudospiralis* and *T. britovi* can survive freezing temperatures);

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<sup>8</sup> OJ L 338, 22.12.2005, p. 27

<sup>9</sup> OJ L 338, 22.12.2005, p. 83.

<sup>10</sup> OJ L 338, 22.12.2005, p. 60.

- The use of the trichoscopic method for examining meat samples is no longer allowed, because it fails to detect *T. pseudospiralis*. A transitional arrangement for four years will give the competent authority the possibility to switch to a more reliable examination method. A number of additional requirements have to be applied whenever the trichoscopic method is used;
- The most important regulatory change is the introduction of *Trichinella*-free holdings or category of holdings or regions having a negligible prevalence. The competent authority can recognise a holding as free from *Trichinella* following an on-site inspection. Animals coming from a *Trichinella*-free holding are exempted from examination for *Trichinella*. The derogation applies only to fattening pigs. Inspection procedures can be very much simplified when the competent authority decides to recognise a category of holdings as free from *Trichinella*. Finally, the draft Regulation provides the possibility for a Member State to declare a region as having a negligible prevalence for *Trichinella*. Third countries will be able to apply the derogation of declaring a holding as free from *Trichinella* as well.

#### 8.4. Regulation on microbiological criteria for foodstuffs

Previously existing microbiological criteria were reviewed taking into account recent developments in food microbiology and scientific advice from the European Food Safety Authority (EFSA). Commission Regulation (EC) No 2073/2005<sup>11</sup> revised these criteria and introduced additional ones. The main objectives of the Commission Regulation are to ensure a high level of consumer protection with regard to food safety and to harmonise the microbiological criteria in the Member States. In particular, the target of the Commission Regulation is to reduce the number of *Salmonella* and *Listeria* cases in humans. A main component of the Regulation is to set two different types of criteria for foodstuffs, which need to be complied with by the food business operator:

- A food safety criterion defining safety of a product or a batch applicable to products placed on the market
- A process hygiene criterion indicating the correct functioning of the manufacturing process.

Microbiological criteria have been laid down for certain micro-organisms which are common causes of foodborne diseases in humans, such as *Salmonella*, *Listeria*, *E. coli*, toxins produced by *Staphylococci* bacteria and histamine. If food safety criteria are exceeded, the batch has to be withdrawn from the market. Food safety criteria have been set for the following combinations of food category/micro-organism:

- A *Listeria* criterion for all ready-to-eat foods
- A *Salmonella* criterion for certain ready-to-eat foods, minced meat, meat preparations and meat products
- A criterion for staphylococcal toxins in certain types of cheeses and milk powder
- An *Enterobacteriaceae* criterion for dried infant formulae
- An *E. coli* criterion in live bivalve molluscs
- A histamine criterion for fishery products

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<sup>11</sup> OJ L 338, 22.12.2005, p. 1.

In addition, the Commission Regulation includes process hygiene criteria, such as *Enterobacteriaceae* and *Salmonella* in carcasses of slaughtered animals, *Staphylococci* in certain types of cheese, *E. coli* in pre-cut fruit and vegetables.

### **Guidance documents**

A number of guidance documents have been prepared to assist the food business operators and the competent authorities of the Member States with the implementation of the Hygiene Regulations (those with an \* have been placed on the DG SANCO internet site):

- Guidance document on Regulation (EC) No 852/2004\*
- Guidance document on Regulation (EC) No 853/2004\*
- Guidance document on the implementation of HACCP and facilitation of the implementation of the HACCP principles in certain food businesses\*
- Guidance document on community guides to good practice\*
- Guidance document on import requirements\*
- Guidance document on the preparation of multi-annual control plans as laid down in Regulation (EC) No 882/2004 (will be published as a Commission Decision)
- Guidance document laying down criteria for the conduct of audits (published as Commission Decision 2006/677/EC<sup>12</sup>)
- Guidance document on official controls, under Regulation (EC) No 882/2004, concerning microbiological sampling and testing of foodstuffs\*.

### **Future aspects**

#### *10.1. Treatment to remove surface contamination*

Article 3(2) of Regulation (EC) No 853/2004 provides a legal basis to permit a substance other than potable water to remove surface contamination from products of animal origin. Such a legal basis did not exist in the previous legislation (Directive 64/433 for red meat, Directive 71/118 for poultry meat, other Directives used to cross reference to the first mentioned), but is available now that Regulation (EC) No 853/2004 is applicable.

With the adoption of the hygiene package and the introduction of the HACCP principles in the entire food chain, establishments are obliged to improve their hygiene and processing procedures. Under such circumstances the use of substances to remove surface contamination of food of animal origin can be reconsidered. It is essential that a fully integrated control programme is applied throughout the entire food chain including on-farm, during transport and in the processing plant. Treatment to remove surface contamination might constitute a useful element in further reducing the number of pathogens, especially with regard to *Salmonella* and *Campylobacter*, provided an integrated control strategy is applied throughout the entire food chain. A draft Commission Regulation has been prepared to allow the use of a number of approved substances for the removal of surface contamination from poultry meat. The draft Commission Regulation is still under discussion.

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<sup>12</sup> OJ L 278, 10.10.2006, p. 15.



### *10.2. Risk-based meat inspection*

Meat inspection has focused traditionally on the detection of the major zoonotic diseases occurring in domestic animals, such as tuberculosis, trichinellosis, cysticercosis, etc. In order to detect these diseases it was necessary to palpate and incise various parts of each slaughtered animal. However, these diseases have either been largely eradicated from herds kept under modern management conditions or do not occur in the majority of the very young and generally healthy animals slaughtered nowadays. Moreover, it has been shown that meat inspection is in some specific cases not the most sensitive way to detect infestation (e.g. in the case of cysticercosis). Furthermore, micro-organisms that are of increasing zoonotic importance in modern animal husbandry systems, like *Salmonella* and *Campylobacter*, are readily transmitted from one carcase to the next by the various manipulations required to be performed during the traditional meat inspection procedures. Taking all these factors into account, a detailed visual inspection without any incision or palpation of slaughter animals might be sufficient to ensure food safety. However, under those circumstances it will be necessary to take efficient preventive measures during the rearing of the animals and to provide sufficient information to the slaughterhouse on the life history of the animals. A draft Commission Regulation has been prepared, which lays down detailed requirements for risk-based meat inspection of fattening pigs and young ruminants.

## THREATS AND NEW TRENDS IN PREVENTING EPIZOOTIC DISEASES IN LIVESTOCK AND POULTRY IN THE EUROPEAN UNION

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### INTRODUCTION

Important changes occurred in the European Union (EU) in the recent past years. The enlargement of the EU to 27 Member States (MS) does not facilitate the conditions of management of the livestock health status and increase the risk of introduction of a major epizootic agent; indeed, the length of the new borders of the EU, the increase of the trade flow associated with the increase of the number of MSs induce more difficulties to implement efficient control measures for the prevention of the onset of epizootic diseases.

#### I) RECENT CHANGES LEADING TO AN INCREASE THREAT FOR (RE) APPEARANCE OF EPIZOOTIC DISEASES

Numerous factors have to be taken into account to assess the risk for the emergence of an epizootic disease.

##### 1) Legal and illegal movements of live animals and products of animal origin

Although it is not possible to establish, in absolute terms, the extent to which the current controls on declared imports have prevented the introduction of animal diseases in the Community, there is a consensus that the overall EU procedures and requirements for declared commercial imports from third countries have been effective and that without the current import controls, there would have been more outbreaks of serious animal diseases (Evaluation Report, 2006). Nevertheless, undeclared and fraudulent trade has been identified as an important and largely unaddressed issue. The recent Foot and Mouth disease epizooty in UK in 2001 seems to be related to the illegal use of swill in a pig farm coming from an Asian restaurant. The subtype of the virus isolated allows assuming that an illegal import of food from animal origin could have been the cause of the introduction of the virus (Gibbens et al, 2001).

- **In regard to the highly pathogenic avian *Influenza* virus (HPAI) H5N1, the legal or informal trade of live domestic birds and, may be; of poultry products is certainly a major factor of introducing a virus when appropriate measures were not taken.** The ban of any import in E.U. seems to have been efficient.
- **illegal trade** is certainly a key route of introduction of the H5N1 virus in countries none previously infected. The *International Herald Tribune* (14/04/06: Promed 136) reported that in Italy, police seized avian poultry products introduced illegally. Tons of poultry products coming from China were also seized by the Dutch customs. For many experts, the origin of the African epizooty is related to the import of infected chickens in one flock in Nigeria. The epizooty was limited to poultry farms and did not affect wild birds.

It is obvious that, in the framework of the World Trade Organisation (WTO), the flow of imported items (food, live animals) increased in the EU these last decades. Meat and animal products originating from infected animals imported illegally probably pose a greater risk to EU than imports from countries with an established and regulated meat trade with EU.

Specifically, there is an increasing trade-driven movement of livestock commodities from FMD-endemic areas in Asia. The supply demand gradient for livestock commodities in these regions seems to gravitate towards either Europe or countries in EU neighbouring regions (North Africa, Middle East). For example, commercial exports of pork and beef by respectively China and India cover the Eurasian zone, including countries which are close to the EU including pork meat to Moldova (4,000 tons in 2004), Ukraine (8,000 tons), Albania (3,500 tons) and beef from India to Georgia (12,600 tons in 2004) and Turkey (2,300 tons) (EFSA opinion AHAW Panel, 2006).

With possible further trade liberalisation as a result of the current Doha Round of WTO trade negotiations, the prospect for increased trade volumes in meat and meat products may bring more challenges to safeguarding animal health status within the EU.

**Thus, there will be a continuing tension between trade policy objectives and animal health objectives which will increase the need for a more risk based approach to border inspections as well as for shifting responsibility and improving risk management at third country level.** (Evaluation Report, 2006)

## 2) Evolution of human behaviour and food consumption

Human populations are moving more and more in the framework of migrations resulting from high levels of poverty in third countries and from wars, but also from tourism and easier travelling conditions. When immigrants are well integrated in one E.U. MS, relatives visit them frequently from their country of origin and take with them traditional food to offer to their family. These cultural and cooking specificities create risks as it induces often illegal even marginal introduction of unexpected hazards. There is a steady flow of small quantities (about 5 kg on average) of animal product being brought in by 1% to 5% of travellers from Asia or Africa. As far as FMD-endemic areas, largest numbers of passengers enter the EU on flights from the far East and from the Middle East and Near East (EFSA opinion, 2006). Assuming only 1% of the travellers bring in an average of 5 kg of animal product given the millions of travellers originating from these areas, this may amount to some 2,000 tons of animal product per year.

## 3) Wild fauna

Several infectious diseases have emerged in the last few decades:

– The **Highly Pathogenic Avian Influenza Virus H5N1** is spreading all around the world since 1996. From the recent events occurred in the world and in the E.U., it can be assumed that **3 routes are responsible of the introduction of the A.I. virus in a naïve country**; among these three routes (including legal and illegal trade), the wild birds following migratory or non migratory routes as, obviously, it happened inside the E.U was the route of introduction of the virus. The aquatic wild birds seem to play a major role in this introduction of the virus. Aquatic wild birds are obviously healthy carriers even if several key points have to be elucidated such as species involved, duration of virus persistence and excretion.

– **Nipah virus**

From 1998 to 1999, a new highly contagious respiratory and neurological disease of pigs was reported on the Malaysian peninsula. There was a simultaneous epidemic of viral encephalitis among employees on affected pig farms and abattoirs. A novel paramyxovirus, distinct from Hendra virus was isolated from both porcine and human victims and named Nipah virus (Bengis et al, 2004). Evidence from virological and serological techniques implicated fruit bats of the genus *Pteropus* as the natural host and reservoir of the virus.

– **West Nile Virus (WNV) infection**

Since 1999, WNV has emerged from North America presenting a threat to human and equine health as well as the health of certain wild bird populations—WNV a well known flavivirus of Europe, Western Asia and Africa, which is maintained in a wide species range of wild birds and birds feeding mosquitoes. Between 2002–2003, the WNV infection was detected in more than 4,000 horses in USA, of which some 20% developed neurological disease (Bengis et al, 2004).

– **Classical Swine Fever (CSF)**

In several MSs of the EU, wild boars appear as the reservoir of the CSF virus in spite of the eradication of the infection in the domestic pig population in most of the EU MSs. The transmission of the virus between wild boars and pigs can occur through direct contacts between these animals, the wild boars being attracted by sows in oestrus when no double fences were put in place to prevent these direct contacts (Artois et al, 2006). But, it seems highly likely that in numerous cases, illegal distribution of swill from wild boar waste could be done to domestic pigs.

#### **4) The climatic changes and the global warming**

– **Example of the Blue-Tongue (BT)**

In 1998, the BT virus (Orbivirus from the family of *Reoviridae*) appeared again in Western Europe after several decades of absence. The recent epizooty differs from the previous transitory appearance of the BT viruses. Indeed, the BT is now present since 9 years in the Mediterranean basin. 8 serotypes of the BTV have been implicated in the different epizooties observed in the South of the EU. The last introductions (including BTV8 with a probable subsaharian origin) came from the south: South Africa (BTV8) and the East (via Turkey). The main vector is a midge named *Culicoides imicola* which is found more and more in northern countries (Toussaint et al, 2006). The global climatic changes and, more specifically, the climatic warming, have an influence on the adaptation and the increase capacity of vectors increasing the risk of appearance of new vector borne diseases.

The same applies to other diseases such as water borne diseases or parasites (AFSSA Report, 2005).

#### **5) Evolution of the farming structure and of the herds management**

For the last 3 decades, under the social pressure, the demand from consumers and economical factors, in several types of production (poultry, pigs, goats...), two models of farming are often opposed with a certain confusion of risk factors associated with the onset and the spreading of infectious diseases.

Recent events with HPAI H5N1, epizooty highlighted the increased risk of contamination related to outdoors farming allowing more frequent contacts with wild birds. It is obvious particularly in Asia, when aquatic domestic and wild birds are sharing the same pools.

Indeed, it is therefore likely that high levels of infection in clinically normal domestic ducks were an important factor that contributed to the epidemic, owing the widespread “seeding” of the virus in countries such as Thailand, Vietnam and Southern China, where ducks are commonly present on farms and range freely on ponds and rice paddies. (Sims et al., 2005)

Always, in regard to HPAI H5N1, at the opposite of the previous model, the industrial indoors farming allows, in an easier way, to implement efficient bio-security measures for preventing contamination. It is obvious that such a situation could protect against HPAI the industrial sector in Thailand where efficient bio-safety measures were put in place.

But, if the protection measures fail with a delayed detection of the first outbreak, it is obvious that the high number of animals present at the same place, combined with a high density of farms, favour a quick spreading of highly contagious viruses with a multiplication of outbreaks. Such factors were involved in the HPAI H7N7 epizooty which occurred in Netherlands in 2003.

### **Conclusion**

The previous examples show that the EU is faced to new challenges and new threats. For preventing the introduction of a pathogen or the emergence of known or new hazards, the implementation of a set of measures and tools adapted to these new challenges and risks will be absolutely necessary.

## **II) CONDITIONS FOR PREVENTION OF THE ONSET OF AN EPIZOOTY**

### **1) Preventive measures**

These measures will depend on the epidemiological situation of the infectious agent to be controlled: enzootic or absence

#### **– Movements of the animals and trade**

Several recommendations which are all essential for the future of the animal health status have been elaborated by the consortium in the framework of the evaluation of the CAHP: Community Animal Health Policy (Evaluation report, 2006):

To reduce the movement of live animals within the Community

To increase and reinforce the Border Inspection Posts Controls (BIPs)

An approach based on three pillars appears to be more appropriate than the present ones involving: greater emphasis on risk analysis and profiling risk based border controls; strengthening cooperation between custom authorities and veterinary services; harmonising the operation of BiPs across the Community.

#### **– Bio-security**

This is a key issue for the future. Whatever the type of farming, it is essential to improve the implementation of bio-security measures in all the farms and to sensitise the farmers about the importance of their implementation. For outdoors and indoors herds, it is essential to prevent any

introduction of agents through passive vectors: boots, straw, wheels, trucks, clothes). For outdoors herds, it is essential to implement systems allowing preventing any contact with wild birds and wild fauna: nets, double fence, winter “gardens”... It seems also essential to impose a minimum distance between two different herds to limit the airborne transmission.

#### – The vaccines

A new generation of vaccines has appeared allowing to differentiate vaccinated from infected animals (D.I.V.A.). These vaccines with companion diagnosis tests can be used to control: Foot and Mouth Disease (FMD), Classical Swine Fever (CSF), Aujeszky's disease (AD), Avian *Influenza*, Infectious Bovine Rhinotracheitis (IBR), (Vannier et al, 2007).

In spite of the major progress these marker vaccines are inducing, it would be a mistake to consider that their use could simply replace sanitary prophylactic measures. Indeed, past experience is very useful to assess the limits and the advantages of the use of these marker vaccines, which could be a powerful tool in a set of measures to control and eradicate a contagious disease. However, the use of such vaccines has to be adapted to the epidemiological situation, the contagiousness of the disease concerned and to the presence or absence of conditions with the capacity to control the spread of infection. To control a disease, the key point is to detect clinically unapparent infected animals (healthy carriers) which can infect in-contact susceptible animals. When vaccination is used, the critical stage of alert induced by the appearance of clinical signs is removed or suppressed. For this reason, such vaccines have to be as efficient as possible not only to protect vaccinated animals against clinical signs, but also to prevent, as far as possible, the excretion of the virus by vaccinated and subsequently infected animals. Moreover, the sensitivity of the diagnostic kits should be as high as possible to reduce to the greatest possible extent, the probability of false negative results; indeed, in such a strategy, the epidemiological consequences of false positive results are less significant than false negative results as positive results are generally confirmed, in a second, complimentary phase, by a reference laboratory using another diagnosis tool.

The longest experience about the use of marker vaccines has been accumulated in relation to the control and eradication of **Aujeszky's disease**. In this case, the use of deleted marker vaccines has represented a considerable advance in programmes to control Aujeszky's disease in several countries.

First, these vaccines have made mass vaccination possible, whilst retaining the means for serological detection of infection. This has enabled vaccinated herds which subsequently become infected to be pinpointed so that the necessary measures can be applied to prevent the field virus from spreading further.

Second, it has become possible to implement sanitary measures in a gradual manner in vaccinated, infected herds, by culling the infected sows at varying speeds, as required. These infected sows were detected through serological screening using the ELISA technique, which enabled vaccinated pigs to be distinguished from those that have been vaccinated and then subsequently infected.

This means that vaccination has a combined effect which allows a programme of prophylactic treatment to be carried out in total safety. Mass vaccination, conducted several years in succession, limits the quantity of virus shed into the air by the infected pigs, thereby considerably reducing the probability and scale of the air-borne spread of contagion between herds (100, 142). Furthermore, systematic vaccination avoids economic losses due to a poorly controlled infection. Consequently, after several years of vaccination in a country or region, the prevalence of infection

gradually diminishes by introducing sanitation measures into the infected herds and continually culling the oldest infected sows; also, the incidence of infection remains very low and is kept under control. However, the cost of vaccination must be taken into account when calculating the total cost of a prophylactic treatment. Where the prevalence of infection in a given territory is high, or there is a high density of pig herds, mass vaccination with effective deleted vaccines is the only means of reducing prevalence; however, although these measures are necessary, they are not in themselves sufficient to eradicate the infection. Identification, screening and culling of the infected breeding animals appear to be essential to successful eradication whilst continuing to systematically vaccinate the animals at least 2 years after elimination of the last infected pig. In the latter case, it is advisable to control the movements of piglets, pigs for consumption and breeding animals as much as possible.

At the opposite, and independently of the performances of the vaccines and the companion kits (which are key issues to determine the use of such tools), other examples show that the use of marker vaccines would not have changed in depth the control of the situation. Following the serious **Classical Swine Fever** epizootic that hit several European countries in 1997, many people believe that the use of these new generation serological marker vaccines could prevent a further animal health catastrophe. An analysis of the situation that existed when the first CSF outbreaks appeared in the Netherlands revealed that more than 22 herds were already infected when the primary outbreak was identified in the region of Venhorst on 4 February 1997. The situation rapidly became dramatic for the region because farmers had already sold piglets before the veterinary administration could isolate the infected zone. This led to a rapid spread of the infection in the south of the country.

Under such circumstances, the use of a serological marker vaccine would not radically alter the basic nature of the problem, as it does not obviate the need for intervention on potentially infected animals, to identify them, to take a sample of serum before any animals are transported, in other words, to strictly control the movement of pigs. Indeed, at the start of an epizootic, the success of control measures depends on their being rapidly implemented after the appearance of the first outbreak and before extensive, undetected spread has occurred. Vaccination is no substitute for basic measures to control contagious diseases

At the start of an epizootic, in regions with a high density of pig herds, ring or zonal vaccination can also be envisaged in order to prevent the virus from replicating too rapidly and to limit the cost of preventive slaughter. However, in this case, transmission of the virus must be limited and control measures must be properly applied and effective.

Such an approach is particularly pertinent for highly contagious diseases such as **Foot and Mouth Disease** in those circumstances under which the airborne transmission is one of the main epidemiological factors for spreading of the virus. So, if the first outbreaks appear in an area with a high density of susceptible herds, under epidemiological conditions that favour airborne spread, a ring vaccination, decided on the basis of the results of models and assessment to determine the risks and directions of spreading, could be useful to try to limit the speed and the extent of the dissemination of the virus. However, due to the ability of vaccination to mask the appearance of clinical signs without preventing infection, vaccinated herds, even with a serological monitoring programme, represent a greater risk for undetected spread than unvaccinated herds, where monitoring can be based on clinical inspection alone.

A successful programme can be based on vaccination, but should also include sanitary measures. Furthermore, when vaccination is part of a control programme, it should be implemented only for a certain period of time. Most of the time, when the prevalence of the

infection decreased significantly and when the epidemiological unit is correctly protected from outside introduction of the agent, vaccination should be replaced by sanitary measures.

Oral vaccination of wild fauna proved to be very effective against **rabies** as it led to the eradication of rabies in fox population in the Western Europe (Brochier et al, 1996). More contrasted results were obtained on wild boars for Classical Swine Fever. Improvement of the tools seems to be necessary.

## **2) Early warning system: detection and prophylactic measures**

The detection of the first outbreak in the first few hours after the infection of the herd by newly introduced agent is the key element determining the control (or not) of the spreading of the infection.

A set of tools and measures is needed to fulfil such a requirement which request considerable means as well an organisation in a country as a structure of the state services which are not found in developing countries.

So, different requirements have to be fulfilled such as:

- active and passive surveillance in the wild fauna, if it is involved in the risk of contamination of the livestock as it is the case for HPAI,
- an appropriate information and sensitisation of the farmers and the veterinarians,
- the existence of an efficient network of field veterinarians owing to an early warning of competent authorities in case of a suspicion of an outbreak,
- the existence of a system allowing a rapid compensation of the losses of the farmers in case of an outbreak,
- an early notification of an outbreak,
- the existence of an efficient and competent network of diagnosis laboratories including an easy and quick transportation of samples from the field to the laboratories,
- the existence of structured veterinarian official services associated with an organized state having a real power of decision with the tools of action to implement effective control of measures including stamping out, control of movements, and assessment of the efficiency of the measures decided...

These conditions are essential to allow an effective control of the spreading of an infection. The onset of an epizooty or the quick disappearance of the infection will depend on the precocity of the detection of the first outbreak (Index case) and on the quickness and the strength of the measures taken by the competent authority.

## **GENERAL CONCLUSION**

Thanks to the knowledge acquired from the past and the onset of modern vaccinal and diagnosis tools, a set of measures can be implemented to prevent or to react when a highly contagious agent, mainly virus, is introduced in the EU. For most of the known agents, these tools and adapted measures appear as sufficiently efficient to allow a quick eradication of the infection. But, in the past, in spite of the existence of efficient tools, dramatic epizooties occurred and can be considered as a failure of this apparatus. Causes of this failure are described in this text. Causes of the adverse events occurring in the EU should be systematically analysed to allow implementing appropriate measures to prevent their reoccurrence.



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## NEW TRENDS IN ANIMAL WELFARE

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### ABSTRACT

Animal Hygiene is, in the words of the organisers of this congress, “a unique scientific interdisciplinary sphere where health and welfare of animals and humans are closely intertwined, and the skills of the discipline are in service of sustainable animal production, public health and biosecurity.” Animal Welfare is, of course, one of the important intertwined disciplines. This is an excellent definition, so far as it goes, but it does not go far enough. Animal Welfare must be viewed not only as a science, but also as a set of values. Moreover, it is not enough simply to consider these subjects as topics for scientific study and moral debate. Right thought alone is not enough. We who are professionally involved have also a scientific and moral obligation to right action: a responsible commitment to promote the health and welfare of our fellow humans and the other sentient animals whom we choose to eat.

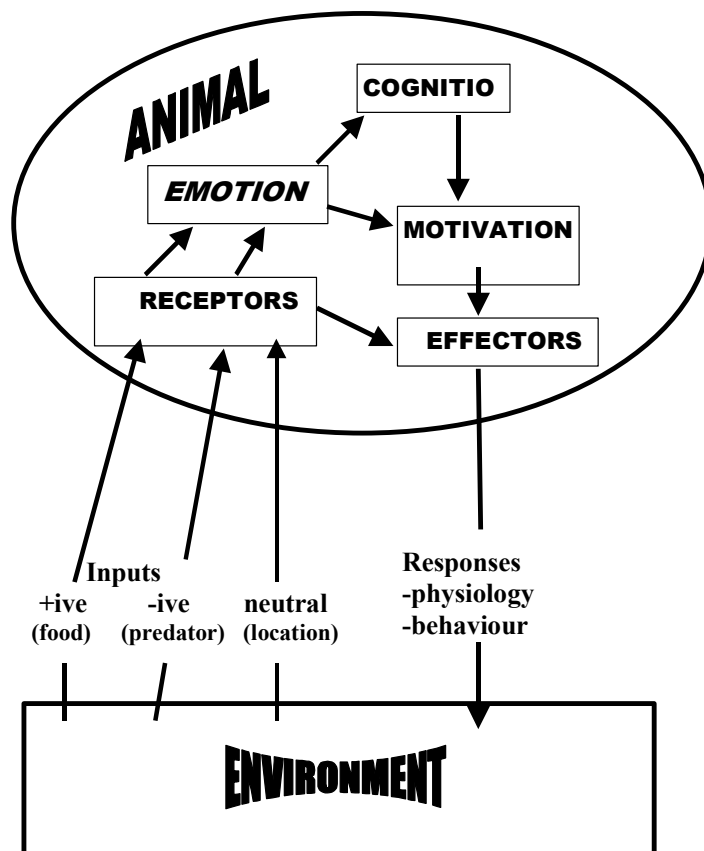
An essay on “New Trends in Animal Welfare” must not, therefore, restrict itself to a review of welfare science. It must also examine the ethical and sociological principles that determine the attitudes of society (producers and consumers) to animal welfare, and explore how the welfare of the food animals may be ensured and improved in ways that are compatible with the other intertwined needs of society for biosecurity, freedom of choice and food at a fair price. This paper will briefly touch on three themes, which are central to the mission of the large, multidisciplinary, multinational ‘Welfare Quality’ programme currently funded by the European Commission under FP6.

- **Animal welfare science:** What is welfare? – What is sentience? – What is suffering? – How can we assess good and bad welfare in a sentient animal?
- **Ethics and values:** What determines human attitudes to animal welfare? – What *should* determine human attitudes to animal welfare
- **Animal welfare promotion:** Welfare monitoring on-farm. – animal welfare promotion through legislation and ‘politics by other means’ – the ‘Virtuous Bicycle’.

### SENTIENCE, WELFARE AND WELLBEING

The most useful definition of welfare, in my opinion, is a personal modification of that of Fraser and Broom (1990), namely “*a state of body and mind as the sentient animal attempts to cope with its environment*”. The two critical words in this definition are *sentient* and  *coping*. This definition covers the full spectrum of welfare from pain to pleasure. We need therefore a separate definition to define good welfare, or *wellbeing*. Here, my simple definition of wellbeing is “*fit and happy*” or “*fit and feeling good*” for those uncomfortable with the word happy (Webster 2005). This too is a state of body and mind. For the body it implies sustained health; for the mind it implies, at least, an absence of suffering from such things as pain, fear and exhaustion. Ideally it should embrace a sense of positive wellbeing (*feeling good*) achieved by such things as comfort, companionship and security.

It is also necessary to have a clear understanding of the word sentience, not least because sheep and other farm animals have been formally recognised by the European Community as sentient creatures, (without ever defining what they mean by the word). My definition of sentience is “*feelings that matter*” (Webster 2005). Briefly, the concept of sentience derives from the way such animals interpret stimuli and sensations, act upon them and review their actions. Consciously perceived stimuli from the external and internal environment are interpreted primarily in an emotional way (“Does this make me feel good, bad or indifferent”). This emotional interpretation may or may not be reinforced by cognition (reason). The emotional (and also, perhaps rational) interpretation of sensation may motivate the animal to action designed to make it feel good, or avoid feeling bad. Alternatively it may deem the information as unimportant and do nothing. The sentient animal will then review the consequences of its action. If it was effective, it will feel better and it will gain the assurance that it knows what to do next time. If its action fails, either because the stress was too great, or because it was constrained in such a way that it was unable to do what it felt necessary in order to cope, then it is likely to feel worse and be more anxious for the future. Thus a sentient animal leads a considered life; its mood and understanding are modified in the light of experience.



**Figure 1.** Sentience: An emotional view of the environment

## STRESS AND SUFFERING

It is necessary to make a clear distinction between stress and suffering as experienced by a sentient animal. When welfare is defined as “*a state of body and mind as the sentient animal attempts to cope with its environment*”, suffering may be defined as its state of body and mind when it fails to cope (or has extreme difficulty in coping with) its environment. When an animal is acutely exposed to a physical or emotional challenge it first experiences a general, non-specific, alarm response involving the hypothalamus/pituitary/adrenal (HPA) axis, which then proceeds to a phase of adaptation. If the stress is moderate, then adaptation may be effective and, in some case, complete. In short, the animal ‘copes’. When the challenge is more intense or more prolonged, the animal may achieve partial adaptation but at some continuing metabolic and emotional cost. If this stress is too intense or prolonged and the cost is too great the animal may proceed to a state of physical and/or mental exhaustion. The definition of *suffering* therefore becomes a state of body and mind where an animal cannot cope (or has difficulty in coping) with physical stress and unpleasant feelings. Extensively reared grazing animals may experience physical suffering through failure to cope with chronic malnutrition and climatic stress a challenging environment. Intensively reared pigs and poultry in crowded barren environments may suffer the emotional consequences of their inability to perform actions necessary to meet their behavioural needs. Fig 2 illustrates the consequences of failing to cope with fearful stimuli. Fear is an adaptive response, essential to survival. Failure to cope with fear may lead to chronic abnormal states such as anxiety or learned helplessness. Suffering, in a sentient animal, is usually a learnt experience. The message for producers and legislators is that some stress in animals is unavoidable. What we must seek to avoid is suffering. Many potential causes of suffering through cruel acts and omissions should be self-evident. Other may be less obvious to a human mind and need the help of animal welfare science to help us understand how sentient animals feel as they seek to meet their physiological and behavioural needs. Thus the aim of welfare science should be to identify and quantify the physiological and emotional challenges to farm animals and devise ways to reduce these challenges and/or help then animals to cope.

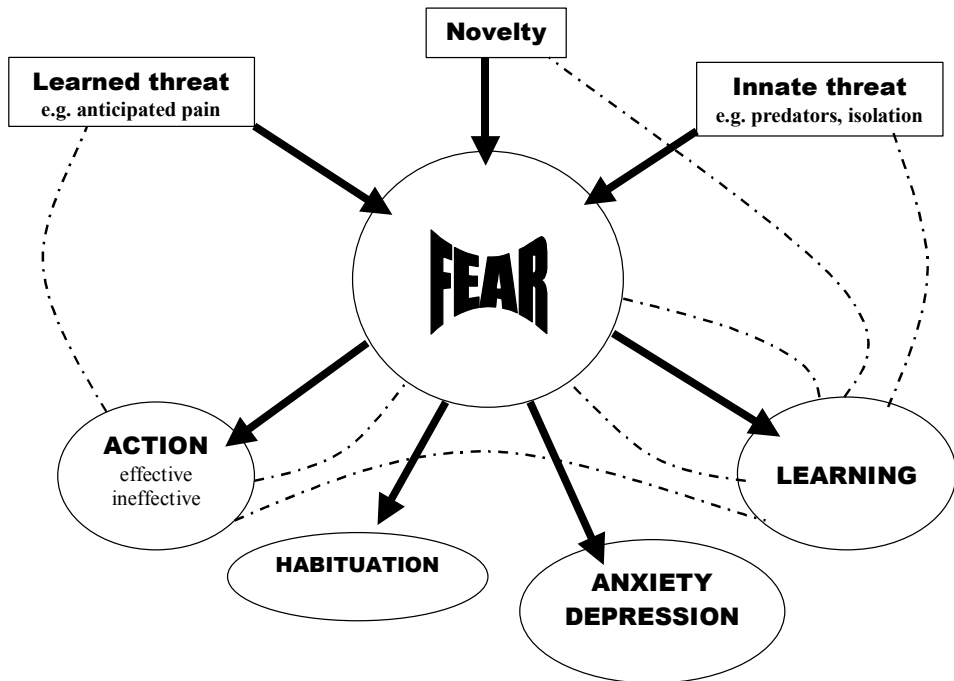


Figure 2. Fear and its consequences

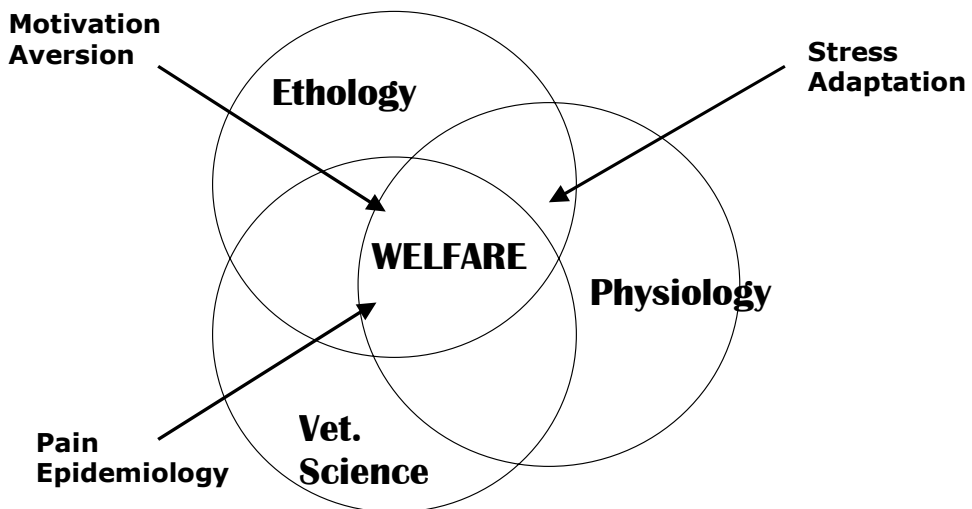


Figure 3. Welfare science as the overlapping element of three scientific disciplines, ethology, physiology and veterinary science

## TRENDS IN ANIMAL WELFARE SCIENCE

Strictly speaking, animal welfare science is not a discipline as such, but the (large) area of overlap between three distinct disciplines: physiology, ethology, and veterinary science (Fig. 3). It follows therefore that a review of new trends in welfare science should highlight new and relevant approaches within these disciplines (and also draw attention to approaches that have become stale and derivative). There is not scope within this paper for a proper review. I shall only pick out some highlights and lowlights.

The science of ethology began with the study of the normal behaviour of animals (the ethogram), first in their natural environment, and then in the presence of environment challenges or environmental restrictions that challenged their ability to cope. This provided convincing evidence that many intensive farming systems were profoundly unnatural and could lead to severe distortions of natural behaviour such as stereotypes. I suggest however that this approach is nearing its “sell-by date”, partly because papers are becoming increasingly derivative but mainly because observations of animal behaviour do not directly address the more important question, which is “how do animals feel?” Much more valuable, in my opinion, is the study of motivation in relation to animal fitness and animal welfare as pioneered by Marian Dawkins (1990). For a recent review of just how far this science has travelled I recommend the review by Kirkden and Pajor (2006). This work addresses the very nature of sentience, pleasure and suffering since it reveals the feelings that matter, measures how much they matter and points to how things may be improved.

The most important (and thoroughly studied) area of overlap between physiology and welfare science is the study of stress. As indicated earlier, the response of animals under stress can be divided into three phases; alarm, adaptation and exhaustion. Much (too much) attention has been directed at trying to quantify the intensity of the alarm reaction by measuring elements of the HPA axis, (e.g. cortisol). There are two critical limitations to this approach. First, the alarm response is non-specific, and does not necessarily distinguish between unpleasant distress and pleasant excitement. Secondly, stress does not equate with suffering and most suffering arises from the cost of adaptation (or exhaustion) after the alarm phase is over. I strongly advise therefore that future studies in stress physiology should concentrate on stress-specific ways of measuring the costs of adaptation, e.g.:

- Physiological costs: increased metabolic rate, loss of body condition, immuno-suppression, exhaustion
- Psychological costs: anxiety, learned helplessness, chronic pain, malaise

The overlap between veterinary and welfare sciences occurs when veterinary science addresses problems of animal health that clearly give rise to a great deal of suffering, where the overall magnitude of the problem is a function of its severity and duration in each individual and its prevalence in the population. By these criteria, lameness is the most severe ‘veterinary’ welfare problem in most farm species (broiler chickens, dairy cattle, sows, sheep). Lameness in broilers is most prevalent in fast growing strains and could be greatly reduced through effective government action to prohibit the sale of the most susceptible strains. In dairy cattle the risk factors are multiple and vary greatly from farm to farm. The most effective approach to investigation and resolution of lameness in dairy cattle is through properly planned investigations carried out not in the laboratory but in the field, with the farm itself being the primary source of input variables. Such experiments are likely to require the active participation of 60 or more farmers and their veterinarians, and this can present a whole new range of problems.

One final point: Welfare science is a “fuzzy” science. Single questions, however elegant and precise, can never yield complete answers. Neither can any of the single disciplines, ethology, physiology, or veterinary science, yield complete answers when studied in isolation. Welfare science should be approached using the old sailor’s technique of triangulation, seeking an approximate fix on the point of interest from at least three directions, in order to minimise the ‘triangle of uncertainty’.

## ETHICS AND VALUES

This argument starts from two fundamental principles.

- We humans have a moral right to rear other species for the production of food.
- Most of the animals that we farm for food are sentient creatures with the capacity to experience well-being and suffering.

Our aim must be to seek an ethical compromise between these two things. Mepham (1996) has devised an “Ethical Matrix”, which identifies the concerned parties whose interests command respect in relation to a specific issue, then applies the ethical principles of beneficence, autonomy and justice to each of the affected interest groups. Here, the interest groups are humans, (consumers and producers), farm animals and the living environment. Humans are the ‘moral agents’, who bear the responsibility for right action; farm animals and the environment are the ‘patients’, profoundly affected by the moral quality of our decisions but unable to contribute to them. Table 1 summarises the rights of all parties worthy of respect, and the responsibilities, which are borne by the moral agents only. Consumers have a right to healthy, wholesome food, and the right to freedom of choice, whether this be governed by price, convenience, taste, appearance, animal welfare or any combination of these and other factors. These rights bring the responsibility to respect the rights of the animals we use for food. In many cases these responsibilities need to be enshrined in legislation, since we all need the help of the state to keep us good (viz. speed limits for motorists).

Farmers have the right to earn a fair living from the rearing of animals in a manner that is efficient, healthy and does not compromise their welfare. These rights should not be eroded by unfair competition, especially when this is imposed by competition elsewhere in the food supply chain. With these rights comes the responsibility to promote animal health and welfare through good husbandry. The simple application of utilitarian ethics should acknowledge that food animals have the right to good health and welfare whether on farm, in transit or at the point of slaughter. A more sympathetic concern for the autonomy of each individual animal should give respect to freedom of choice, best achieved through environmental enrichment. Justice for the farm animals requires that they should experience “a life worth living”.

Any moral view of the production of food from animals should also embrace a proper respect for the living environment. In this regard, farms should not be viewed simply as food factories, but as one of the most powerful forces for good or bad in relation to environmental quality. Farmers who own the land now are the stewards of the land for all of us, for ever. We are justified in criticising them if they destroy the habitat of wildlife or pollute the rivers. However we cannot expect them to sustain and enrich the quality of the living environment simply on the money that we (the consumers) pay them for producing food as a commodity. If we wish to ‘save the planet’ then we must all make our contribution. As always, we shall need some help from legislation. One of the more promising new trends in this regard is the evolution of the EC Common

Agriculture Policy (CAP) to encourage and reward Environmental Stewardship Schemes, which recognise the need of society to contribute, through taxation, to the cost of sustaining the quality of the living countryside.

**Table 1.** Application of the ethical matrix to identify rights and responsibilities in relation to the farming of animals for food

	Wellbeing	Autonomy	Justice
Human society (Consumers)	Healthy, wholesome, tasty food, fairly priced	Freedom of choice	Respect for animals enshrined in legislation
Producers	Financial success	Free competition	Good husbandry for animals and the land
Farm animals	Wellbeing on farm, in transport and preslaughter	Environmental enrichment	“A life worth living”
Living environment	Conservation and sustainability	Biodiversity	Respect for the environment and its stewards

### **RIGHT ACTION: PROMOTION OF ANIMAL WELFARE**

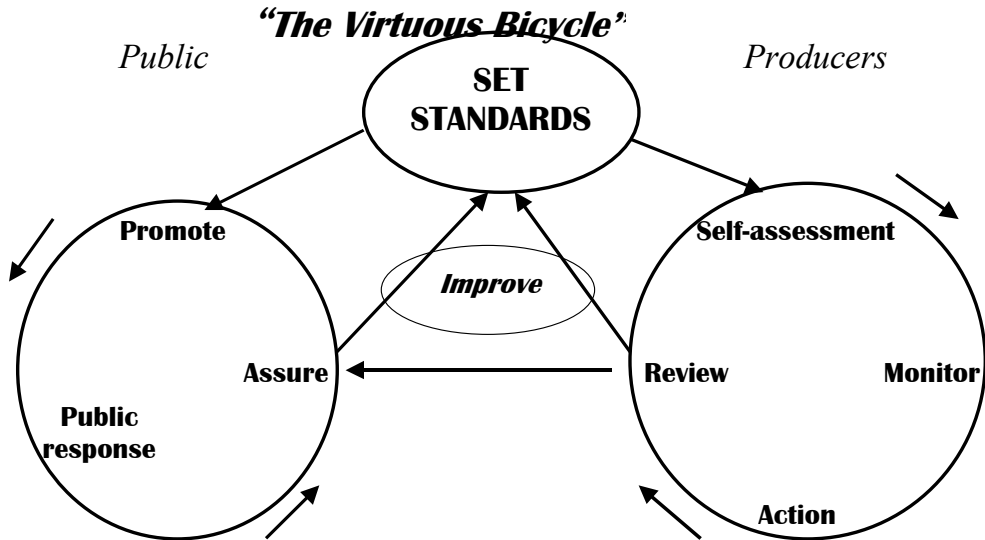
The drive towards improved welfare for the farm animals should be driven by four engines for change, all operating together.

- Increased international awareness of the nature of animal sentience and the responsibilities that this entails.
- Realistic, practical, step-by-step, strategies for improving animal welfare consistent with efficient, economic production of safe food from healthy animals.
- Legislation to encourage and enforce improvements to farm animal welfare.
- Increased consumer demand for ‘added value’ foods, where animal welfare is an essential and proven area of added value.

The first action, increasing awareness, is perhaps the most important of all. Too many people, in too many regions of the world, are simply not aware of the nature of sentience and suffering in farm animals. Once they are, their attitudes should improve (if only a bit). Welfare charities like CIWF, RSPCA and WSPA have demonstrated that they are the most effective media for spreading this awareness. The second action, the development of effective strategies for improving farm animal welfare, depends on continued progress in our understanding of what it takes to keep farm animals fit and happy. and the application and marketing of these principles through the coupled virtuous cycles of quality assurance and quality control (Fig. 4).



## Welfare-based Quality Assurance



**Figure 4.** ‘The Virtuous Bicycle’: coupled progression to monitor, ensure and promote high standards of animal welfare

### WELFARE-BASED QUALITY ASSURANCE

Wherever shoppers for food are offered a choice and have a reasonable income, they demand quality. They can set their own standards for qualities such as appearance, taste and price. However they have to take other things on trust, such as source, food safety, and production standards which, of course, include animal welfare. This has generated a plethora of farm assurance schemes ranging (in U.K.) from the ‘Little Red Tractor’ to organic standards set by the Soil Association and ‘Freedom Food’ welfare standards set by RSPCA. The intention is that both consumers and producers should benefit from a system that adds value based on the quality of the production methods. Organic food standards (which include a proper concern for animal welfare) have been conspicuously successful. As a general rule however, it is probably unrealistic to expect that animal welfare, considered in isolation, will be sufficient to attract affluent, choosy consumers looking for added value. It is more likely to succeed when incorporated into a package that incorporates other selling points such as local, sustainable and fair trade. The notable exception to this general rule is free-range egg production according to the ‘Freedom Foods’ standards that now make up about half of total egg sales in many U.K. Supermarkets.

The most important question for professionals and indeed the animals is ‘Do these welfare-assurance schemes deliver what they claim to deliver?’ Do they:

- Ensure good standards of animal welfare?
- Ensure better standards of animal welfare than on unassured farms?

- Address specific welfare problems as they occur?
- Incorporate a protocol for regular review and upgrading of standards?

At present, the answer to all these questions is either ‘No’ or ‘Don’t know’. Most current standards are based on measures of the resources and records necessary to promote good husbandry. This is good in so far as it goes but it fails to address the most important questions ‘Are the animals fit and how do they feel?’ At Bristol, my colleagues David Main, Becky Whay and I have developed animal-based protocols for the direct assessment of animal welfare outcomes (see Main et al. 2003, Webster et al., 2004, Whay et al. 2003a, b). To summarise our published and unpublished work very briefly I can say that the welfare of the free-range hens in our study, in general, looked good but dairy cows had their problems, especially lameness whether or not the farms were accredited to Freedom Foods or Organic standards. The need to incorporate direct, animal-based measures of welfare into Quality Assurance schemes has been recognised and taken up by the FP6 ‘Welfare Quality’ programme.

One of the main problems with Farm Assurance schemes is that they can simply become pieces of paper to be filed away between inspections. A scheme for Farm Animal Health and Welfare only becomes effective if it is part of a dynamic strategy to ensure and improve standards. This is illustrated in Fig. 4. The accreditation body sets husbandry and welfare standards acceptable to both producers and consumers/retailers. The sequence of events for the producer is as follows. S/he first carries out a self-assessment of the enterprise to check on compliance with standards and identify any problems. An independent monitor then assesses the unit using a protocol looking mainly at welfare outcomes. Farmer, monitor and veterinary surgeon then address any immediate problems and devise a living strategy for health and welfare. The effectiveness of this strategy is reviewed after an appropriate time (e.g. one year or less if there are problems that need to be resolved quickly). The effectiveness of the strategy then feeds back to the farmer for further self-assessment and to the accreditation body who can bench-mark the farm against approved standards and provide real assurance to the public as to what is being done. This sets in motion a virtuous cycle of review, action, improvement and further review.

Any welfare-assurance scheme will, of course, only work if the public is aware of it, value its standards and trust the assurances that it provides. It is necessary therefore also to set in motion a second virtuous cycle of information transfer between the accreditation authority and the public that sets out clearly the quality standards and provides honest evidence to indicate how well the scheme is working. Simultaneous rotation of both wheels creates a ‘Virtuous Bicycle’ which can bring benefits to all concerned parties; consumers, producers, and the animals themselves.

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## **PARALLEL SESSIONS A1, A2, A3**

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### **A1 – IMPACT OF ENVIRONMENT ON CATTLE BEHAVIOUR, HEALTH AND PERFORMANCE**



# ORAL PRESENTATIONS

## IMPACT ON AND DEMANDS FOR HEALTH AND WELFARE OF RANGE BEEF CATTLE

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### SUMMARY

There is uncertainty about to what extent and on which way ranch operation can and should be applied in order to guarantee cattle health and welfare during the climatic conditions in Sweden. An account for the current knowledge is compiled from a comprehensive literature review and own experiences from systematically performed herd studies. As conclusion recommendations are presented for animal management and for design and management of pastures and buildings.

**Keywords:** beef cattle; range husbandry; cattle health; calf mortality; weather resistance

### INTRODUCTION

Beef cattle herds are kept extensively outdoors all the year round in parts of Canada, the US and some European countries where the climate during winter might put a great strain on the animals. There is uncertainty about to what extent and on which way ranch operation can and should be applied in order to guarantee cattle health and welfare during the climatic conditions in Sweden. The aim with this paper is to give an answer to these questions.

### METHODS

The study consists of a thorough and comprehensive literature review, an account of own experiences from systematically performed herd studies and herd investigations in Sweden and elaboration of recommendations based on thus obtained knowledge.

### RESULTS AND DISCUSSION

#### ***Cattle physiology and behaviour of importance at range management.***

The thermoneutral zone refers to the temperature interval within which the animal's heat production is independent of the temperature in its environment. When the environmental temperature is lower than the thermoneutral zone the lower critical temperature (LCT) is attained. Then the animal must increase its heat production in order to maintain constant body temperature. Ruminants then must redistribute their energy to heat regulation (Senft and Rittenhouse, 1985).

Similarly, when the environmental temperature exceeds the upper critical temperature the animal must decrease its heat production. LCT is partly depending on the animal's insulation, coat, subcutaneous fat etc., and partly on the animal's heat production in the thermoneutral zone.

Webster (1974) states LCT for an adult beef cow to be  $-13^{\circ}\text{C}$  at cloudy and calm weather and  $-3^{\circ}\text{C}$  at cloudy weather and wind velocity of 4,5 m/s provided that the animals have dry lying area. LCT for beef cattle with summer coat, or wet winter coat, is estimated to be  $15^{\circ}\text{C}$ , with autumn coat  $7.2^{\circ}\text{C}$ , with winter coat  $0^{\circ}\text{C}$  and with thick winter coat  $-7.8^{\circ}\text{C}$  (Wagner 1988).

Cattle undergo habituation on cold dependant upon how long time the exposition has lasted. Cattle must be exposed to cold during at least one week before the habituation process will start (Christopherson and Young 1986). Habituation does not occur after a short exposure to cold or by intermittent exposures during short periods (Kennedy et al 2005).

Cattle habituate to cold from summer to winter through growth of coat and establishing of a subcutaneous fat layer whereby the insulation increases, thus making it possible to maintain the body temperature unchanged. Experimental studies in climate chambers have shown that cattle shiver when exposed to  $-20^{\circ}\text{C}$  in September but not in December which indicates that the coat has adapted to cold during the autumn (Gonyou et al 1979).

Acute cold stress is met with shivering, muscle trembling with a frequency of 10 per second, whereby heat energy is released (Andersson & Jonasson 1993).

There are examples of adaptation to cold through genetic selection. Ice bear is an example within the family bears, and arctic fox within the family foxes.

Also within species differences exist. The coat structure differs between Aberdeen Angus and Hereford. However, the two coat types have the same insulation capacity (Gilbert & Bailey 1991).

Experimental studies of dry respectively wet coat show that wet coat substantially increases the heat loss (e.g. Hillman et al 1989; Jiang et al 2005). While the heat losses from a dry coat are negligible those from a wet can amount to 200–300 W/m<sup>2</sup> (Cena & Monteith 1975). Studies of the insulating capacity of reindeer coat at different coat humidity and different wind velocities show that the impact by humidity from mist or easy rains did not change the insulating capacity. However, heavy rains significantly reduce the insulating capacity of the coat through evaporation and by heat-conducting water replacing the insulating layer of air in the coat (Cuyler & Øritsland 1999, 2004).

New-born calves are noticeably tolerant towards cold at dry and calm weather provided they are given dry bedded lying areas (Ekesbo 1963; Radostits et al 1999). Calves kept in hutches cope with temperatures between  $-8^{\circ}\text{C}$  and  $-30^{\circ}\text{C}$  on condition that the straw bedding is dry (Rawson et al 1988, 1989a, b).

Adult cattle with experience of being kept outdoors the year around seek shelter to a greater extent than younger against adverse weather conditions (Beaver & Olson 1997). This seem to be an at least partly acquired behaviour explaining the observation that only cattle which have learnt to use sheds seek shelter in those against adverse weather (Ekesbo 2006). Preference tests show that cattle prefer sheds to forests during winter and to lie on straw bedding to the bare ground (Wassmuth et al 1999). Cattle offered free choice between resting and seeking shelter outdoors or in sheds with dry straw bedding choose in winter the shed and in the summer to lie down outdoors (Krohn et al 1992).

Adult cattle on pasture rest lying 10, 1–11, 6 hours per day, calves longer (Krohn & Munksgaard 1993; Albright & Arave 1997; Phillips 2002). Experimental studies show that cattle are inclined to invest a lot of work in order to get possibility to lie down (Jensen et al 2005). Preference tests show that cattle offered wet lying areas have substantially shorter resting period

than if offered dry lying areas (Keys et al 1976). At cold and wet weather cattle also seek for dry lying areas (Wassmuth et al 1999).

Comparison between different bedding materials shows that cattle prefer straw before other materials (Jensen et al 1988). Straw as bedding gives better protection against cold than e.g. wood shavings (Rawson et al 1989a).

### ***Impact on cattle health and behaviour by range keeping.***

Epidemiological studies of cattle kept indoors respectively outdoors show that animals kept outdoors in general have better health (Ekesbo 1966; Bendixen et al 1986a; Wassmuth et al 1999; Wassmuth 2003). When individual herds are compared this general rule is not valid since specific injuries or diseases might exist caused by environmental or management conditions in the single herd.

Regional hypothermia, frostbite, with ensuing necrosis can affect ears, tail and feet of calves (Radostits et al 1999). At pronounced hypothermia the blood flow to peripheral organ such as ears and tail is reduced, the cells rupture and gangrene arises. Eventually the frost bitten tissue will be rejected (Robertshaw 2004). Frostbites on ears, tails and hind legs in new-born calves exposed to cold are described from Canada (Radostits et al 1999) and from Sweden in dairy calves sucked by other calves (Ekesbo 1963). Calves sucking other calves do not seem to occur among calves in ranch cattle herds (Lidfors 1994).

The mortality from birth to weaning varies in different studies from 10.3% to 3.3% (Patterson 1987; Allen & Liénard 1992; Alves et al 1989; Busato et al 1997a; 1997b; Dutil et al 1999 ; USDA 2006; Swedish Dairy association 2006). In a comparison between different breeds in Sweden it varies for calves after heifers between 1.2% and 5.2% and for calves after cows between 1.9% and 7.1% (Swedish Dairy association, 2006).

The most common cause of deaths of new-born calves is complications after difficult calving (Azzam 1993; Kasari 1994; Wikse 1994; Ganaba et alder. 1995; Dutil et alder. 1999). In a study of more than 5000 calvings the mortality after difficult calving was 20, 4% and after normal calving 5, 0% (Laster & Gregory 1973). The differences in mortality depend upon the breed and age of the cow (Ekesbo 1966; Lindhé 1968; Bendixen et al 1986b; Swedish Dairy association, 2006) but also upon the sex of the calf.

There is a clear connection between increased calf mortality and the combination rains, temperature at or under 0°C and wind (Ekesbo 1972; 1973; Martin et al 1975a, b, c).

Muddy and soak surfaces, e.g. around watering-troughs, feeding places and passages constitute risks for hoof diseases (Monrad et al 1983; Manske 2002; Step & Smith 2006).

Covering permanent feeding places with straw has been tried in order to protect the pasture surface against poaching and facilitate gathering of the manure. However, it requires 3, 5 kg straw per grown animal and day (Wassmuth et al 1999).

### ***Impact on feed consumption and growth in adult cattle by weather protection or lack of weather protection;***

The requirement of pasture land per animal at Swedish ranch operations is calculated to vary between 1.5 and 3 hectares (Ekesbo 1991).

Thyroxines are secreted when animals during longer time have been exposed to cold. This increases their appetite and feed requirement (Westra and Christopherson, 1976; Young, 1981). However, the time grazing per day decreases with falling temperature (Malechek & Smith 1976; Christopherson 1983; Adam's et al 1986; Dunn et al 1988; Beverlin et al 1989; Prescott et al 1994). If not supplementary feeding is given in winter when grazing time per day will be reduced,



or when the feed supply is insufficient, the animals therefore must use their energy reserves for maintaining heat balance.

Cattle without sufficient protection against unfavourable weather show increased feed consumption and decreased daily growth (Hoffman & Self 1970; Cunnings et al 1972; Leu et al 1977). Cattle with access to sheds show better daily growth in winter time than those provided only with wind shields (Hoffman & Self 1970; Milligan & Christison 1974; Leu et al 1977). However the quality of the laying area, dry and thereby heat insulating or moist and thereby heat-conducting, has a greater influence on the ability to maintain growth during unfavourable weather than shelter from wind or low temperature (Christopherson 1981; Mossberg 1992).

Preference tests show that cattle in winter choose resting in sheds with dry straw bedding but in summer choose resting outdoors (Krohn et al 1992).

### ***Animal management. Design and management of pastures and animal enclosures;***

Calvings in ranch operations are, for economic reasons, often planned to late winter, early spring, from the month of March. However, this requires rigorous monitoring of the pregnant animals, especially the heifers. Increased risks for calf mortality at calvings early in the year is an important argument for calvings to take place during the hot season (Olson et al 1981a, 1981b; Josey et al 1987; Wittum et al 1990), also in countries milder climates than in north Europe (Rowan 1992).

At the same time as it is well known that cattle prefer to choose secluded and sheltered places for calving studies in Finland show that calving cows more often than not calving cows choose to stay in sheds (Lidfors et al 1994).

Problems with animal losses and inappropriate designed sheds gave rise to experimental wind tunnel studies in Canada aiming at learning how to design sheds in order to give optimum shelter against wind and precipitation (Theakston 1960, 1962; Theakston & Underwood 1961). Also later has these questions been dealt with (Charles 1991).

A cattle herd outdoors does not seek shelter in a shed which one day will be placed in their pasture. Sheds are not included in cattle's evolutionary world of conception. Therefore they must learn that sheds give shelter to adverse weather. To do so a shed should be provided with plenty of straw as bedding. After some time, other bedding materials might be used, e.g. sawdust or wood shavings. However preference tests show that cows prefer straw as bedding before others (Jensen et al 1988). In order to have the animals in future to use the shed the bedding must be carefully looked after and, when necessary, new bedding material supplied thus avoiding the laying area to be moist and wet.

## **CONCLUSIONS**

Based on literature and own studies and investigations in different herds during several years the following conclusions are drawn regarding what to observe in order to avoid health and hygiene problem in cattle ranch operations under the climatic and other conditions in Sweden and comparable countries.

The calving period should be limited to about two months in order to avoid to great age differences between the calves. Separate not too large enclosures for calving facilitate the supervision of the animals at calving, especially if calving occurs early in the year. However, if too many animals are crowded together the pasture surface will be destroyed by poaching and the animals will not get enough secluded areas for calving. In larger herds heifers should have calving

enclosure separated from the cows'. If calving occurs early in the year sheds, preferably open front sheds, with dry bedding for shelter should be available for calving animals.

For animal welfare reasons and in order to minimize the calf mortality difficult deliveries must be given immediate professional assistance. For this specific premises with efficient heating devices must be available as well as suitable transport vehicles to get even lying animal there.

It is in most cases sheds with dry bedding area are required during the cold season in order to give protection against adverse weather. The depth of open front sheds should be not less than 12 m in order to give enough protection. If there is forest around the shed 8m depth might be enough. The free height on the open side should not exceed 4 m. Some breeds, in the first place Highland Cattle, but in certain cases also Hereford and Aberdeen Angus, can manage without sheds if mature spruce forest offers dry laying areas and protection against adverse weather. Only mature coniferous forest is robust enough to stand up to the wear of the animals hoofs. The number animal per area may not exceed 1 per hectares in these cases. In order to give protection against adverse weather in general a forest depth about least 200 m is required.

It should not be underestimated that too many animals in relation to available land area are a risk factor at ranch operations.

The ground on the feeding places must be protected against poaching and manure accumulation when supplementary feed is given. If there is not substantially frost in the ground, the feeding places must therefore be changed daily irrespective of feed is given on the ground or at movable feeding mangers. Permanent feeding places, a deviation from the ranch operation idea, requires concrete or similar surfaces in front of the manger which make it possible to scrape and carry away the manure.

Close to watering places the ground should be well drained and suitably designed in order to prevent hoof damages and diseases.

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## ALLEY-FLOOR DESIGN, CLAW LESIONS AND LOCOMOTION IN SWEDISH LOOSE-HOUSED DAIRY CATTLE

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### SUMMARY

Effects on claw lesions and lameness of rubber vs. mastic-asphalt flooring in solid alleys, scrapers vs. no scrapers on top of slatted concrete alleys, and feed stalls vs. no feed stalls were studied in a 2-yr 2x2 factorial experiment, using 183 Swedish Holstein cows in a research cubicle herd. Most cows were scored as mildly lame at least once, but at the majority of weekly scorings cows were non-lame. Scrapers, as well as rubber comparing with mastic asphalt, reduced the risk of lameness. We found no effects on the risk of claw lesions at the end of the study.

**Keywords:** claw lesion; dairy cattle; feed stall; lameness; locomotion; mastic asphalt flooring; rubber flooring; scrapers

### INTRODUCTION

Concrete is a common material in alleys for loose-housed dairy cattle, presumably due to its durability, low cost and ease of cleaning. However, alley flooring design affects cow behaviour and the risk of claw lesions and lameness. For instance, dairy cattle are able to distinguish walking surfaces differing in traction (Phillips and Morris, 2002) and rubber flooring in front of the feed bunk increases the time cows spend standing in concrete alleys (Fregonesi et al., 2004). Unfortunately, concrete walking surfaces have a negative effect on locomotion (Telezhenko and Bergsten, 2005) and may increase the risk of claw disease and lameness (e.g. Bergsten and Frank, 1996), comparing with more yielding surfaces such as rubber and mastic asphalt. Mastic asphalt is made of acid-resistant bituminous compounds and fine siliceous sand, particle size  $\leq 0.5$  mm (BINAB, NCC Roads AB, Stockholm, Sweden), with a relatively high coefficient of friction. Scrapers of various types are commonly used to clean solid loose-housing alleys. They have also been installed on top of slatted flooring to improve cleanliness further and reduce ammonia emission. Theoretically, scrapers would decrease the risk of infectious claw disease in cows on slatted alleys but, to our knowledge, such an effect has not been studied previously.

Cattle often displace each other while feeding by butting with their heads. Bouissou (1970) tested different partitions between dominant and subordinate cows and found that partitions separating the heads and bodies of the cows increased the feeding time of subordinate individuals. However, studied animals were horned and the results cannot be extrapolated to larger and socially more complex cow groups. Kongaard (1983) suggested that cows might feel more protected when there is a physical barrier between the animals during feeding. In practice, feed stalls are used to reduce social stress and the consequences of agonistic behaviour at the feed-

bunk, thus presumably securing feed intake and milk production, but also to improve standing comfort, foot cleanliness and claw health. DeVries and von Keyserlingk (2006) showed that feed stalls reduce competition at the manger and improve access to feed, especially in subordinate cows. However, we found no previous scientific study of the influence of feed stalls on claw health.

The objective of the present experiment was to study the effect of solid rubber vs. mastic-asphalt flooring in alleys, of scrapers on top of slatted concrete flooring in alleys, and of feed stalls, on claw lesions and lameness in dairy cows housed in cubicle systems.

## MATERIAL AND METHODS

The study was conducted as a double 2x2 factorial experiment during two consecutive housing seasons (2002–2004) at a university dairy farm in southern Sweden. Claw lesions were observed in 75 (year 1) and 115 (year 2) cows of the Swedish Holstein breed, parity 1–5; 30 of the cows participated both years. Locomotion was observed in 96 (year 1) and 121 (year 2) cows; 34 of the cows contributed both years. Seventy-two (year 1) and 92 (year 2) cows were observed for both lesions and locomotion.

All cows were loose-housed in a cubicle system with four equally-sized pens, each subjected to a separate treatment but otherwise identical. In each pen there were 21 cubicles in two rows parallel to the manger, and one computer-controlled concentrate feeding station along the outer wall. The cubicles measured 1.2 x 2.4 m and were equipped with cubicle partitions (Solid, DeLaval, Tumba, Sweden) and 30-mm polymeric mats (Cow Mat CM30L, DeLaval). They were littered with sawdust, providing new litter material twice a week. Alleys between cubicle rows were 2.2 m wide. In each pen there were four water bowls, placed in the cross passages between alleys. The cows were milked twice daily in a 2x9 herringbone parlour. The holding area had solid concrete floor and a mechanical crowd gate, and room for a maximum of 50 cows. The cows walked approximately 30 to 50 m on slatted concrete from their pens to the milking centre. The cows were fed concentrates (grain, soya and supplements) partly in feeding stations, and partly together with roughage (grass-clover silage) in a mix given *ad libitum* at the manger twice daily. Additional hay was fed during a 60-d period in the spring of 2003, when several cows exhibited loose faeces. All cows were grazed daily from beginning of May to beginning of September between the two housing seasons. Claw trimming was performed at the beginning and end of the observation period (in September-January and in January-May). Trimming was done by a third author or a professional claw-trimmer using an electrical grinder and a hydraulic trimming chute.

In year 1, the alley floor was newly installed slatted concrete (single 125-mm concrete beams divided by 40-mm slots) and the treatments were: scrapers (Delta Master DM II automatic hydraulic scrapers with rubber blades, DeLaval, installed on top of the slats) and feed stalls, no scrapers but feed stalls, scrapers but no feed stalls, and neither scrapers on the slats nor feed stalls. In year 2, the alley floor was solid and the treatments were: rubber mat flooring (KURA-P, Gummiwerk Kraiburg Elastik GmbH, Tittmoning, Germany) and feed stalls, 25 mm mastic asphalt flooring and feed stalls, rubber mats but no feed stalls, and mastic asphalt but no feed stalls. To render a comparison between years possible, a fifth treatment (23 cows) in year 2 had concrete slats (4 years old) without scrapers or feed stalls. If present, there were 20 feed stalls per pen, measuring 0.8 x 1.6 (2003–2004) to 1.7 (2002–2003) m, equipped with a standard hard rubber mat (Gummimattan Marianne Larson AB, Gothenburg, Sweden). The alley behind feed stalls was 2.2 to 2.3 m wide, and the alley along the manger without feed stalls was 3.9 m wide.

Treatments were expressed by three dichotomous variables representing scrapers (*No; Yes*) in year 1, type of solid flooring (*Mastic asphalt; Rubber mats*) in year 2 and feed stalls (*No; Yes*) in both years. Cow group (1 group per pen each year) was expressed by a categorical variable. The cows were allocated to treatments (18–23 cows in each pen) in early autumn each year by blocked randomization in order of expected calving (every fourth cow going into a given pen, random order of pens) within parity (primiparous and multiparous), and introduced 4 to 90 d before calving; however, 62 cows that calved before the observation period (<113 d) were allocated in order of observed calving. No cows changed pen during the housing season.

Claw-lesions were recorded in connection with claw trimming at the beginning and end of observations, originally scored on 4-level ordinal or dichotomous scales. One trained recorder performed all examinations. All feet were immobilized at a convenient height and the trimming area was well lit-up to facilitate the assessment of lesions. Clinical diseases and therapeutic measures were recorded successively. Claw-lesion records at second trimming were recoded into four binary outcome traits with a sufficient number of cases and non-cases, representing heel-horn erosion, haemorrhage in the sole or white line (including sole ulcer), dermatitis, and separation in the white line or double sole (0 for score 0 and as 1 for scores >0). Lameness data were collected at 31 and 34 weekly sampling occasions, from September to May. At each occasion, all cows were scored once for lameness by one of three persons (year 1,  $n=1375$ , 435 and 315 records, and year 2,  $n=1618$ , 557 and 79 records, respectively) as the animals walked a 30-m alley (slatted concrete with 125-mm slats and 40-mm slots) from the milking parlour to the cubicle area and while they were standing in front of the manger. Lameness score 0 denoted normal (level-back) posture; cows with score 1 stood with a level-back posture but walked with an arched back; cows with score 2 both stood and walked with an arched-back posture (Sprecher et al., 1997, modified). Lameness score was transformed into a binary trait representing lameness status by changing records with scores  $\geq 1$  to 1.

Analyses were done using generalized linear mixed models in the GLIMMIX procedure of SAS 9 (SAS for Windows, SAS Institute Inc., Cary, NC, USA), assuming a binomial distribution and including group as a random effect. Lesion traits were modeled at the foot level, specifying repeated measures within cow by a compound-symmetry correlation structure (different years within cow assumed independent). Only cows that were exposed for treatment until at least 85 days after calving were used, excluding 104 cows year 1 and 16 cows year 2; cows were exposed for between 107 and 245 days (median 190 days) before lesion assessment at second trimming. In total, 648 observations in 141 cows were used. Lameness was analysed by two different strategies. First, lameness status at each occasion was modeled as separate observations, specifying a first-order auto-regressive correlation structure for repetitions within cow, assuming observations to be decreasingly correlated over time within cow (different years within cow assumed independent). In total, 4359 observations in 168 cows were used. By the second analytical strategy, the portion of observation time lame was modelled. A binary outcome trait was coded as 1 if the percentage of occasions scored as lame was  $\geq 50\%$ , otherwise as 0. In this model, different years in the same cow were assumed independent. Totally 193 observations in 167 cows were used.

Tested fixed-effect predictors at the group level represented year (1; 2) and studied treatments (scrapers [year 1] or solid flooring type [year 2], and feed stalls). Cow-level predictors represented parity, calving season, days in milk at the start of the observation period, days in milk at lesion scoring, days in milk at lameness scoring. Foot-level predictors of lesions represented pair of feet (*hind; front*) and lesion status at first trimming regarding the same type of lesion (0; 1). Occasion-level predictors of lameness (1<sup>st</sup> strategy) tested represented days in trial, days since

last claw trimming, and rater. The final models were built by a manual stepwise procedure, starting with full models. Predictors representing year, either scrapers or solid flooring type (nested within year) and feed stalls were forced into all models; among remaining predictors, only those associating statistically significantly with the outcome (Type 3  $P \leq 0.05$ ) or confounding a treatment effect were retained in the models. Thereafter, biologically plausible 1<sup>st</sup>-order interactions were tested and included if statistically significant (Type 3  $P \leq 0.05$ ). Differences between predicted population margins (least-squares means) of treatments and interacting factors were computed, and corresponding odds ratios and their 95% confidence intervals were calculated.

## RESULTS

Most prevalent lesions were haemorrhage of the sole (67 and 84%), haemorrhage of the white line (43 and 62%), dermatitis (24 and 32%), and white-line separation (21 and 23% of cows affected in one or more feet at the end of the observation period in year 1 and 2, respectively). The prevalence of most lesions was higher in year 2 than in year 1; there were however only two cases of sole ulcer, in year 1. There was no need for any emergency treatments of claw diseases during the study. Most of the cows (58% in year 1 and 70% in year 2) were scored as mildly lame at least once, but the majority of scorings (55% in year 1 and 65% in year 2) were 0. Forty-eight percent of the cows were lame  $\geq 50\%$  of the scoring occasions in year 1, and 39% in year 2. Comparing the scoring of lameness between study years under similar housing conditions, there were 273 observations (54%) scored as 0, 200 (39%) as 1 and 35 (6.9%) as 2 in the treatment without scrapers or feed stalls in year 1, while there were 400 observations (71%) scored as 0, 139 (25%) as 1 and 22 (3.9%) as 2 in the fifth treatment in year 2.

Feed stalls increased the odds of haemorrhage by 1.8 in hind feet ( $P=0.032$ ); otherwise, there were no significant associations between treatments and risk of claw diseases. The correlation between lesion records in two different feet of the same cow was estimated to be from 0.054 (separation) to 0.19 (haemorrhage). The proportions of the total variation in lesions residing at the group and cow levels were 0.6% and 26%, respectively, for the model of heel erosion, 2.2% and 14% for haemorrhage, 4.4% and 14% for dermatitis, and 0.5% and 18% for separation (estimated from empty variance-component models, without fixed effects).

In cows with access to feed stalls, comparing with no feed stalls, the odds of lameness at weekly scorings were 60% lower in second parity ( $P=0.002$ ), but 40% higher in older cows ( $P=0.02$ ); in first parity, the odds were marginally significantly higher (OR=1.6,  $P=0.05$ ). In cows of third or higher parity kept on slatted concrete with scrapers, comparing with no scrapers, the odds of lameness at weekly scorings were 55% lower ( $P<0.0001$ ), but no effect of scrapers was found in younger cows. Likewise, in cows of third or higher parity kept on solid rubber flooring, the odds of lameness were 61% lower ( $P=0.01$ ) than on solid mastic asphalt, while no effect of type of solid flooring was seen in younger cows. There was no significant association between treatments and risk of lameness at  $\geq 50\%$  of the scorings. No significant association between time exposed to treatments and lameness was found. The correlation between to consecutive weekly lameness recordings in a cow was estimated to be 0.48. In the model of weekly recordings, the proportions of the total variation of lameness residing at the group and cow levels were estimated to 0.26% and 62%, respectively; in the model of lameness at least half the time, 0% resided at the group level.



## DISCUSSION

In the present study, the prevalence and severity of claw lesions were very low. Only a few sole ulcers and abscesses were found and there was no need for emergent lesion treatment during the study, although over 100 cows were observed during two years. Possible explanations are extremely good management of the animals, excellent cow comfort through mattresses, very good alley hygiene, and balanced diets for an average production level. Thus, given the circumstances, it was no surprise that significant differences in the risks of lesions between treatments could not be detected in the present study. In the same animal material, Telezhenko et al. (2006) showed that feed stalls on an abrasive floor (mastic asphalt) reduced the weight-bearing area of the sole and thus resulted in a high contact pressure, which might explain the found effect of feed stalls on haemorrhages in the present study. Furthermore, comparisons of claw health between floorings with different abrasiveness might be biased due to different rates of claw horn growth and wear (Telezhenko et al., 2005). Vokey et al. (2001) and Vanegas et al. (2006) revealed positive effects on claw disease and/or lameness of solid rubber alley flooring, comparing with solid concrete. Danish (Thyssen, 1987) and Swedish (Hultgren and Bergsten, 2001) studies have shown that slatted flooring can result in a significantly lower risk of interdigital dermatitis and heel horn erosion than solid flooring.

The scoring system used was based on both direct symptoms of lameness with altered weight bearing and the cows' signs of discomfort when walking, expressed by the arched back. The latter mechanism is very sensitive, i.e. non-lame cows can arch their backs when walking on uncomfortable flooring, like the slatted flooring used here. A majority of animals arched their back when walking, while a minority of animals arched their back while standing. No animals showed any lameness expressed as altered weight bearing. These results are in accordance with the low prevalence of claw lesions. Claw lesions cause most but not all lameness (Murray et al., 1996), and lesions are not necessarily expressed as lameness (Manske et al., 2002).

Although lameness scores were low, animals kept on slatted flooring with scrapers had significantly lower scores than those without scrapers. However, infectious lesions – such as dermatitis and heel horn erosion – were not influenced significantly by type of flooring, which may indicate that locomotion scoring was more sensitive. We also found that solid rubber flooring (in contrast to solid mastic asphalt) decreased the risk of lameness in dairy cattle of parity 3 or higher. This is in accordance with Telezhenko and Bergsten (2005), who found the gait of animals with a high locomotion score to be impaired on hard floors. It was expected that feed stalls with rubber mats would decrease the risk of lameness; however, the increased risk in cows of third or higher parities seems less logical.

We included a fifth treatment in year 2 (slatted floor without scrapers or feed stalls) to be able to make comparisons between years. However, this slatted floor was older and considerably more worn than that used in year 1, which made such direct comparisons difficult. The proportion of cows scored as lame differed considerably between the two treatments (29 and 46%, respectively).

## CONCLUSIONS

No association could be shown between scrapers on top of a concrete slatted floor (comparing with slats without scrapers), a solid rubber floor (comparing with solid mastic asphalt) or feed stalls (comparing with no feed stalls), and the risk of heel-horn erosion, sole or white-line haemorrhage, claw dermatitis, or separation in the white line or sole horn of dairy cattle. Scrapers on top of a concrete slatted floor (comparing with slats without scrapers) and a solid rubber floor (comparing with solid mastic asphalt) decrease the risk of lameness in dairy cattle of parity three or higher – as judged by posture scoring while standing and walking. Feed stalls seem to decrease the risk of lameness in cows of second parity, but not in older cows.

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## THE FEEDING BEHAVIOUR OF DIARY COWS AND THEIR WELFARE

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### SUMMARY

In an intensive production, whole system of feeding is organized around of milking procedure. Diary cows in such circumstance modified their natural feeding activities in the level which farmers allowed them to express, and their instincts in the cases of feeling hungry and for free grazing are changeable in the way of expressing. In that sense, the aim of this study was to show that feeding behaviour of diary cows is under strong influence of breeders. Farmers, with applied technique of feeding can create certain activities of diary cows during the feeding. When the ration of feed is not proper and when feed distribution is not regular, diary cows react with anxiety and frustration. In such cases, welfare of breeding animals is at risk.

**Keywords:** feeding, behaviour, video recording, sequential analyses, diary cows, hunger, welfare

### ORIGINAL ASPECTS OF THE RESEARCH

System of breeding of diary cows is very important because it has influence directly on the level of feed consumption and milk production. In that reason, feeding behaviour of diary cows can help us to understand why different practice of breeding the same species of cattle have different milk production.

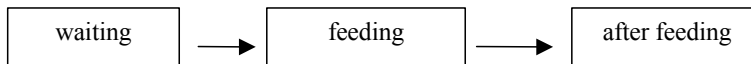
In natural environment cows can always behave according to their instinct of grazing animal and voluntary will when they want take the feed. This instinct is a part of motivational power of natural behaviour of cows. Diary cows in intensive industrial systems are completely deprived of these congenital activities and they have to modify feeding behaviour to special demands of farmers. In such cases, when they feel hungry, animals have to reduce instincts for grazing, because the only that they can do is to wait the feed from farmers. This motivation of waiting for eating in the case of chronic frustration is a special kind of stress feeding and can produce serious digestive disorders.

In this work, basic hypothesis were: that there is a difference between feeding activities in the different systems of breeding; that some activities are repression by the farmer and that this repression has influence on feeding. According to this, the basic hypothesis in this work was that it is possible to create welfare of diary cows in the aspect of feeding behaviour with regular distribution of feed, always the same time-table and with correct amounts of feeds.

## OBJECTIVE OF THE WORK AND METHOD

Research was done in two farms of dairy cows which are different in the way of breeding, free and tied, during three months of examination in the summer period. In the farm with free system of breeding, feeding was one time daily, and in the farm with tied system, feeding was two times daily. The work consisted of video recording of feeding in the following categories: high pregnant heifers, lactating cows and dry cows. Periods of recording with video camera started 15 minutes before feeding and lasted 45 minutes during the feeding. Every category of animals was recorded in several repetitions, by the recommendation of Blackshaw (examination, duration, number and kind of activities). This kind of behaviour measuring is “limited recording of behaviour”; it considers continual observation of the certain activities only. With this method video record could be divided on individual sequences. Each sequence represent particular pattern of activities. The Beckeman’s method was used to define: function of sequence (transport the new information from breeder to the cows), and kind of interaction (giving-taking the feed). The used method of sequential analyses gives the possibilities of creating the schemes of activities of which feeding behaviour consist. The chart 1 shows model of video recording feeding behaviour of diary cows which is used in this work.

Continual observing behavior of living being by the method of sequential analysis can be applied when the researcher wants to keep some aspects of natural appearance in the time function. Also, video record offers more repeating and can give more reliable measuring of activities. That way, the error of human components decreases to minimum. Using the sequential analyses as a method give the possibilities to express activities of behaviour in the numerical data. This data are finally statistically analyzed with analyses of variance and *Tuckey’s honest significant difference test*.



**Chart 1.** *Model of sequential analyses of feeding behaviour used in this work*

We record a large number of activities and because of that we choose by three activities in every sequence. In the sequence of waiting this was activities: standing, watching and mooing. In the sequence of feeding we choose activities: taking the feed, selection and defending from stable fly. In the third sequence this was activities of searching for more feed, rumination and lying. The way how we defined observed activities was that every one be in the function of representing the sequence.

## EXPERIMENTAL DATA AND RESULTS

In this research we made interactions of feeding activities of dairy cows between two experimental farms. The first exam category was high pregnant heifers. Common behaviour characteristic of on the both farms was in the sequence of waiting the ration, where all observed head were standing and watching, expecting the entrance of feed-dispenser. This activity indicates on already created feeding behaviour with farmers training because the high pregnant heifers were learned to the feeding schedule. Maybe the farmers were not quite conscious about the fact that

they on this way created new feeding activities. In captivity systems which are unnatural environment for diary cows, such activities are good example of response in adapt behaviour, because the only allowed activities when they feel hungry (natural this would be grazing) is to stand ready in expecting with a head turning around left and right, watching the entrance in stable. One of visual excitement is seeing feed-dispenser and hearing excitement is the sound of same machine. On both excitement observed animals reacted with anxiety. Moo activity of high pregnant heifers in the sequence of waiting had a statistically important interaction between two farms.

The same pattern in the sequence of waiting represents the source of stress and frustration. In the case where farmers did not give to the observed high pregnant heifers the meal in the learned time for feeding, they reacted with moo, roaring and with anxiety. This reaction was the same in the examination categories of lactating cows and dry cows, as well. We assume that feeling of hunger physiological reinforced the fear and make specific feeding stress. In both farms, during the examination period this was very often (only 5% recording diary cows were feeding in the learned time).

Interesting difference was in the number of activities in high pregnant heifers in defending from the bites of stable flies. High pregnant heifers in the farm with free system had less activity then the same group in the farm with tied system of breeding. This trend during the observation was the same at the lactating cows and dried cows. Cow that was bitten by stable flies stop eating, take of her head from feed-bunk, swing with tail or turn her head left-right drive away the insect and then again return for feeding. On the next stable fly bite cow again stop to eat with the same reaction.

Activity of selection of feed during the last feeding sequence had a statistically very important interaction between examination farms in the all category of observed diary cows (high pregnant heifers, lactating cows, dry cows). In the way of expressing this activity was gradually increased. In the first part of feeding, from the moment of giving the feed to 15 minutes of consumption, between the high pregnant heifers on the different farms is obtain statistically important interaction. After that, in the same feeding sequence, but in the last part of video recording the consumption, from 15 to 30 minutes, in the categories of lactating cows, dried cows and high pregnant heifers, is obtained statistically very important interaction.

We recorded the next pattern of feeding behaviour in the second and third sequences. In the beginning of feeding, cows were concentrate on consumption with choosing the meal activities (pushing with snout), but how the amount was decrease the level of selection for better feeding place get increased. Feeding behavior of diary cows in the farm with tied system in the sequence after feed, (when they eat all feed), was consist from activities of licking the empty crab and licking the feeding alley. In farm with free system, in the feeding sequence diary cows (all category) of breeding were choose the feed with activities of mutual struggle who get increased how the amount of feed decreased. These activities, in two ways, can indicate to the feeding practice. First, when the amount of feed is not sufficient, cows in free system of breeding express competition fight with strong mutual head pushing or with completely body struggle. Stronger animal will secure for herself more feed then the weak one (who produce more milk than dominate in herd). Second, diary cows in tied system of breeding in such cases can develop stereotype activities such as licking the empty feed-bunk and feed alley or licking another animal.

## CONCLUSION

### Practical implication of the work

Motivation for feeding behaviour consists from hunger feeling and appetative behaviour that eliminate this unpleasant feeling. Feel hunger is subjective and each animal experience subjectively but with mutual physiological mechanism and neurology regulation of feeling hungry and satiety. The ways how diary cows express their feeding behaviour are different in the different systems of breeding. Application of sequential analyses offer possibilities to estimate behaviour activities between animals and represent one of the paths for define welfare of the diary cows.

Expressed activities in examined diary cows, in the farm with tied system and farm with free system of breeding, in the sequences of waiting, feeding and after feeding indicated that: before feed distribution cattle were anxious, that when the meal is given diary cows concentrate on taking the feed, and how the amount of feed is decrease they again express anxiety. Method of sequential analyses with video recording can help in feeding behaviour observation.

In this research we made: etograms of feeding activities of diary cows, their interaction between different sequences and their interaction between two experimental farms. It was obtain very small number of statistical high important interaction between two farms and this are: activity of cows moo show high pregnant heifers and activity of feed selection in the sequence of feeding at high pregnant heifers, lactating cows and diary cows. The others observed activities did not give interaction between different systems of industrial breeding.

These results can show that feeding behaviour of dairy cows is strongly influenced by breeder. Farmer, with amount of feed offered and with the way of distribution can make particular good or bad activities of feeding behaviour and on that way he has influence on welfare of dairy cows. Definition by Broom that “welfare of one individual is her state in the her ability to adjust to the environment” clearly show to us how important role of breeder is and his influence on welfare of dairy cows by proper feeding which can help animals to avoid stress of captivity.

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## CLAW LESIONS OF THE DAIRY CATTLE IN TABRIZ AREA IN IRAN

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### ABSTRACT

Claw disorders cause 90% of lameness in dairy cattle. Factors that have been associated with claw lesions are individual factors like stage of lactation, parity, weight and genetics. Herd factors involved are housing, environment, management and nutrition.

Infectious claw lesions like dermatitis and heel horn erosions are mainly influenced by the environment. Laminitis or claw horn disruption has largely been attributed to feeding regimens and especially rations high in rapidly fermentable carbohydrates have been focused on.

In this study the trial was performed on dairy farm located in Tabriz (Iran). The target population of this study consisted of 200 dairy cattle. Hooves were evaluated for incidence of hoof disease. Heel erosions, hemorrhages and digital dermatitis were the most prevalent hoof problems. A total of 41% of hooves were determined to be Unhealthy.

Overgrowth of the horn is a result of environmental factors exacerbated by predisposing disease factors. Functional hoof trimming restores the normal shape of the claw, the angle of the toe and equalizes the weight distribution between the two claws.

### INTRODUCTION

Lameness and claw-health are of increasing importance in herd-health management. Lameness is an increasing problem, comparing recent studies on the prevalence of lameness in dairy cows (6). According to the 2002 National Animal Health Monitoring Systems (NAHMS) survey, 16% of cattle are culled due to lameness. However, this survey may underestimate this number as cows culled for low production (19%) or reproductive failure (27%) may actually have been lame. Lameness has been shown to reduce milk production (3) and fertility (7). Infectious claw lesions like dermatitis and heel horn erosions are mainly influenced by the environment. Laminitis or claw horn disruption has largely been attributed to feeding regimens and especially rations high in rapidly fermentable carbohydrates have been focused on. Epidemiological research on claw disorders in dairy cattle indicates that the main infectious claw diseases resulting in hoof lesions and lameness are digital and interdigital dermatitis (6). Since the use of antibiotics in footbaths is banned, many farmers are advised to use chemical disinfectants in footbaths like Copper Sulphate, Zinc Sulphate, formalin and their combinations for the prevention and treatment of (infectious) claw-problems. But, some of the contributing factors are nutrition, hygiene, cow comfort (freestall management), walking surfaces, time spent standing on concrete, hoof health, and claw trimming. The objective of this study was to determine the incidence of claw lesions in of dairy cattle in Tabriz area in Iran.



## MATERIAL AND METHOD

The trial was performed on a dairy farm located in Tabriz area, in Iran. The target population of this study consisted of 200 dairy cows, Holstein breed that were milked twice a day. The housing consisted of free stalls, cleaned every two days and concrete floors. The foot bath solution used prior to the trial consisted of 12 kilograms of copper sulfate diluted in 50Gal of water and it was located in the alley at the exit of the parlor, preceded by a wash bath containing fresh water, the cows walked through the foot bath solution once a day in the week. Weather during the trial had the average sunny days. Hooves were evaluated for incidence of hoof disease. In the cases, hooves affected by Heel Erosion, Hemorrhages, Digital dermatitis, Foot rot, White line disease and Ulcers were trimmed and management. In this cases, were walked through the foot bath solution twice days in the week for 1 month.

## RESULTS

Heel erosions, hemorrhages and digital dermatitis were the most prevalent hoof problems. A total of 41% of hooves were determined to be unhealthy. These results are summarized in the table 1.

**Table 1.** Initial Examination.

Disease	Number	%
Heel Erosion	35	<b>17.5</b>
Hemorrhages	20	<b>10.0</b>
Digital dermatitis	13	<b>6.5</b>
Foot rot	1	<b>0.5</b>
White line disease	2	<b>1.0</b>
Ulcer	11	<b>5.5</b>
Healthy hooves	118	<b>59.0</b>
Total	<b>200</b>	<b>100</b>

In affected cases, after 1 month, the hooves were examined again. Only, a total of 20.5% of hooves were determined to be unhealthy and 79.5% of hooves were determined to be healthy. After functional hoof trimming, 50% of unhealthy hooves were treatment after 1 month. These results are summarized in the table 2.

**Table 2.** Examination after 1 month.

Disease	Number	%
Heel Erosion	25	<b>12.5</b>
Hemorrhages	3	<b>1.5</b>
Digital dermatitis	10	<b>5</b>
Foot rot	1	<b>0.5</b>
White line disease	0	<b>0</b>
Ulcer	2	<b>1</b>
Healthy hooves	159	<b>79.5</b>
Total	<b>200</b>	<b>100</b>

## DISCUSSION

Sub solar hemorrhaging, White line disease, and sole ulcers are primary indicators of a previous laminitis. While a nutrition factor like rumen acidosis seems to be a key in the development of laminitis, different observations suggest that additional factors must be involved (2). In a 1989/90 survey, around 20% of healthy dairy cows had their claws trimmed to prevent lameness (5). Around 36% of the cows' claws were trimmed by farmers, 31% by contractors, 23% by veterinary practitioners and 10% by students and others. In a study of 15 dairy farms, Mill and Ward (1994) found that only 20% of farmers had their cows' feet trimmed regularly (4). It is recommended that foot trimming is carried out at least once a year after housing, or preferably twice a year before and after housing (1). In a 1980 survey of farmers in England and Wales, 2% of farms were found to have footbaths (8). Around 56% of the footbaths had been installed to cure rather than prevent lameness. Most farmers used 1.6% to 4% formaldehyde. Frequency of use of the footbaths ranged from once a day to less than once a fortnight. It is recommended that dairy cows are walked through a footbath containing 2% formaldehyde (5% formalin) or 5% copper sulphate once a week and no more than twice a week (1). Twice daily cleaning of cubicles and passageways of cattle houses to reduce faecal contamination will help to prevent foot lameness. However, the benefits of this expenditure of time are not specific to lameness. Changes to housing, milking parlour, yard and race design, floors and roadways, and bedding may reduce the incidence of lameness. No data are available on improvements made to housing, yards and roadways with the specific intention of preventing cases of lameness. In this study, foot bath and trimming helped control a range of hoof problems when used in a well maintained foot bath. Overgrowth of the horn is a result of environmental factors exacerbated by predisposing disease factors (2). Functional hoof trimming restores the normal shape of the claw, the angle of the toe and equalizes the weight distribution between the two claws.

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## EFFECT OF WINTER HOUSING FACILITIES ON ANIMAL HYGIENE, SOMATIC CELL SCORE AND MASTITIS INCIDENCE

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### SUMMARY

Three designs of wood-chip out-wintering pad (OWP) (sheltered, unsheltered and OWP with silage provided on top of the woodchips) were compared to indoor cubicle accommodation with regard to cow cleanliness, somatic cell score (SCS) and mastitis incidence. Cows on OWPs were accommodated at a stocking density of 14.52m<sup>2</sup> per head. Although sheltered cows were cleaner than cows on the unsheltered OWPs, there was no difference in SCS or mastitis incidence between treatments. Accommodating animals on OWPs at this stocking density during the winter poses no greater threat to udder health than housing indoors in cubicles.

**Keywords:** dairy cow, animal hygiene, mastitis, somatic cell count

### INTRODUCTION

Mastitis is one of the main production diseases of dairy cows. The disease leads to reduced milk production (Rajala-Schult, 1999), increased costs due to prevention and treatment (Kaneene and Scott-Hurd, 1990), and may ultimately result in the culling of animals (Grohn, et al., 2005). Unhygienic accommodation systems have a negative impact on udder health (Bartlett et al., 1992), so in developing alternative accommodation systems it is important to consider the potential for maintaining a clean environment. Outwintering pads (OWP's) are a low cost winter accommodation system for cattle that may be a suitable alternative to indoor cubicle housing for dairy cows in Ireland. They consist of a woodchip lying area, with or without shelter, which has a drainage system underneath. Grass silage can either be conserved on top of the woodchips to facilitate self-feeding, or fed from an adjacent concrete feed face. Under optimum management conditions the woodchips are replaced when they become soiled. OWPs pose no threat to the health and welfare of beef cattle (Hickey et al, 2002). However, previous work showed that housing spring calving dairy cows on an OWP at a high stocking density (6m<sup>2</sup> woodchip area/head) during the winter leads to higher dirt scores while on the OWP, and reduced udder health three weeks post-calving compared to cows in cubicles or on an OWP with a lower stocking density (12m<sup>2</sup> woodchip area/head) (O'Driscoll et al., 2006). Results from the latter were confounded by the fact that cows accommodated at the high stocking density had shelter, while cows at the low stocking density did not. The objective of this study was to compare three OWP options for spring calving dairy cows that incorporate various design features (concrete feed face vs. self-feeding on the woodchips; sheltered vs. unsheltered) with traditional indoor cubicle

housing. Stocking density was the same on all OWP's. The measures chosen for analysis were dirtiness scores, incidence of mastitis, and somatic cell scores (SCS).

## MATERIALS AND METHODS

### *Animals and treatments*

Ninety six pregnant dairy cows (*Bos Taurus*) (40 primiparous and 56 multiparous) were blocked according to breed [Holstein-Friesian or Norwegian Red], parity ( $1.56 \pm 1.785$ ), expected calving date (ECD) (22 Feb 2006  $\pm$  20.1 days) and body condition score ( $3.09 \pm 0.29$ ) into 8 groups. Animals were randomly assigned to one of the following treatments; (i) indoor cubicles (IC), (ii) an unsheltered OWP with a concrete feed face (UP), (iii) a sheltered OWP with a concrete feed face (SP) and an unsheltered OWP with a self feed silage pit (SF) in two replicates. Multiparous and primiparous animals were assigned to treatment on 17 November 2005, and 5 December 2005, respectively.

Indoors the cubicles were bedded with rubber mats and provided at a ratio of 1:1. They were of a 'Super Dutch Comfort' design (O'Connell, et al., 1991) and were manually cleaned and treated with lime each day. Animals on UP and SP were allocated 12m<sup>2</sup> woodchip space allowance per head and a concrete feed face allowance of 2.52m<sup>2</sup> per head (40cm per cow). SF cows had a woodchip space allowance of 14.52m<sup>2</sup> per head. IC cows were also fed silage from a concrete feed face with 40cm per cow. The feed face of IC, UP and SP was cleaned 6 times daily by an automatic scraper. The feed faces in each SF replicate were 13.5m in length. In order to prevent spoilage of the silage, it was necessary for 26 animals to feed from these areas. For this reason 14 'filler' cows were allocated to each of the SF replicates. OWPs were scored weekly for cleanliness using a subjective 4 point scoring scale (1=fresh woodchip, 4=extremely soiled woodchip), and managed so that each animal had a clean lying area of 2.2m<sup>2</sup>/head.

All multiparous cows were dried off by 17 November 2005. The mean calving date was 21 February 2006. Approximately 3 days pre-calving ( $3.2 \pm 5.51$  days) animals were removed to a straw bedded calving shed. Cows remained with the calf until the next milking, after which they returned to a separate straw bedded shed for one night. Thereafter, cows that calved between 24<sup>th</sup> January and 13<sup>th</sup> February 2006 (n=40) were kept on a separate unsheltered OWP by night and were at pasture by day, until 14<sup>th</sup> February, when they were kept at pasture both day and night. Cows that calved from 14<sup>th</sup> February onwards were at pasture by day and night. As cows calved and were turned out to pasture the stocking density in each treatment was maintained by adjusting an electric wire (UP, SP and SF) or metal gate (IC) accordingly. Cows were re-randomised and accommodated on either an unsheltered OWP (n=36) or at pasture (n=60) from 18 April which was the start of the breeding season, for 6 weeks after which they all returned to pasture until the following November. All cows were dried off by 19 December 2006.

Grass silage was offered *ad-libitum* daily in the morning at 0.1kg above requirement in order to ensure animals were not restricted. Fresh water was available from self-filling troughs in each treatment. While at pasture animals were managed as a single herd. Pasture consisted primarily of perennial ryegrass (*Loilum penne*). (Animals that were accommodated on UP during the breeding season were offered freshly cut grass *ad-libitum* each morning).

### ***Animal dirtiness scoring***

Animals were scored for dirtiness at the beginning of the experiment, and subsequently approximately every 2.5 weeks ( $17 \pm 3.3$  days) until 30 January. Only animals that had not yet calved and were still in their treatment groups were scored. The animal was scored on the left side of the body, which was subdivided into 5 areas; front leg, rear leg, hind quarter, belly and udder, and each section scored using a scale from 1 (clean) to 4 (completely soiled) in half score increments. The sum of these scores constituted the overall dirtiness score for each animal (max = 20, min = 5). The scoring system was tested for inter and intra observer repeatability prior to the experiment.

### ***Somatic cell count (SCC)***

Cows were milked twice daily for the entire lactation, at approximately 07:00 and 15:30. Milk yield was measured at each milking for every cow using electronic milk meters. Clusters removed automatically once milk flow fell below 0.2kg/min. Individual cow milk samples were taken approx. every other week ( $15.4 \pm 5.84$  days) at one morning milking, and SCC determined using the Bentley 300 (Bentley Instruments Incorporated, USA). The last sample to be included in the analysis was taken on 1 November 2006. On average  $14.4 \pm 3.48$  samples were taken per animal.

### ***Clinical mastitis (CM)***

Clinical mastitis was assessed daily by the stock people over the period from assignment to winter accommodation, and the following inter-calving period. The udder was observed for redness, soreness, and/or inflammation as indicators of CM. On identification of a case of CM a sample of milk from the affected quarter was taken aseptically and analysed for bacteriology. These recorded cases are referred to as CM, and are based solely on the herdsman's interpretation of CM (Pryce et. al., 1999).

### ***Quarter milk samples (QMS)***

Quarter milk samples were taken at drying off (30 November 2005), approx. 3 weeks post partum ( $18.3 \pm 3.52$  days), and on 14 June 2006. All samples were analysed for bacteriology as well as SCC quantification. Quarter milk samples were also collected  $1.8 \pm 1.29$  days post calving, and assayed for California Mastitis Test (CMT) and bacteriology. On each QMS test day all milk samples were collected aseptically from all udder quarters into individual sterile plastic containers after drawing of foremilk. Clinical mastitis was diagnosed at each QMS test day when macroscopic changes in the milk or udder were observed. Subclinical mastitis was diagnosed at each QMS examination when  $SCC > 500,000$ ,  $CMT > 2$ , and no macroscopic changes were evident.

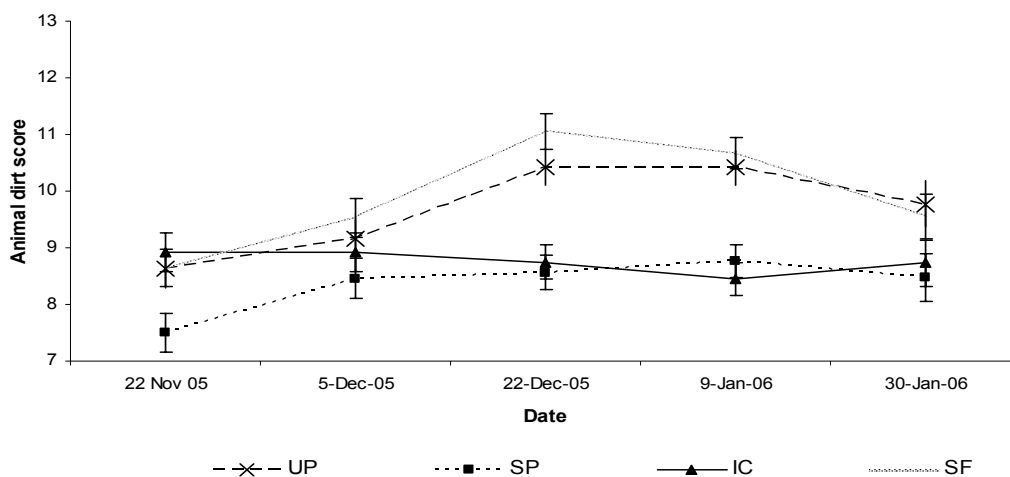
### ***Statistical analysis***

All data were analysed using the Statistical Analyses System (SAS, V9.1). The animal was considered the experimental unit. Data was tested for normality prior to analysis. A  $\log_2$  transformation of SCC to SCS was used to normalize the data distribution. Dirt scores were analyzed using repeated measures, with inspection day as the repeated variable using the MIXED procedure. Treatment and inspection day were considered fixed effects. Replicate was considered a random effect. The interaction between treatment and inspection date was also examined.

Lactation average SCS was calculated as the mean of all SCS test day records for each cow within the lactation. Average SCS was also calculated for three stages of lactation: 6 to 60 days in milk (DIM) (early), 61 to 220 DIM (mid), and 221 to 305 DIM (late). Lactation average SCS was analysed using ANOVA with a mixed model. Replicate was included as a random effect. Cow was nested within replicate and treatment. Treatment, whether the animal was accommodated on treatment while lactating, accommodation during the breeding season, lactation number and breed were treated as fixed effects. Average SCS for each stage of lactation was also analysed using a similar model. Residuals were examined to verify normality and homogeneity of variances. Differences in the incidence of CM, SCM, and the incidence of pathogens detected in QMS were analyzed using Fishers exact probability test.

## RESULTS

On January 20<sup>th</sup> 2006 there was less than the recommended clean lying area per cow in all treatments so all OWP's were cleaned and the woodchips were replaced. Treatment had an effect on animal hygiene, SF cows having the highest dirtiness scores overall ( $9.8 \pm 0.27$ , mean  $\pm$  s.e.) and SP cows the lowest ( $8.3 \pm 0.27$ ;  $P < 0.001$ ). There was no difference between SF and UP or between IC and SP ( $P > 0.05$ , data not shown). There was no difference in dirtiness scores between treatments at the initial exam ( $8.3 \pm 0.27$ ;  $P > 0.05$ ), or at the final exam (post cleaning) ( $9.1 \pm 0.18$ ) (Figure 1.). Dirt scores in IC remained at a level similar to the initial exam over the course of the experiment ( $P > 0.05$ ). Cows in SP had numerically the lowest score at the initial exam, and a score similar to IC on all other occasions. However, dirtiness scores in UP and SF increased from the start of the experiment until the OWPs were cleaned.



**Figure 1.** Effect of treatment on the dirtiness scores of cows at five inspections

There was no effect of treatment on average SCS over the entire lactation, or in stages 1, 2 or 3 of lactation ( $P > 0.05$ , data not shown). There was only 1 case of CM during the dry period, and this occurred in SF. There was no difference in the number of animals displaying symptoms of CM at calving or during the lactation period. There was no difference in the number of animals on each

treatment diagnosed with SCM at drying-off, at calving, at the three week post partum exam, or on 14 June, or in the number of animals that had pathogens isolated from QMS on any test day ( $P > 0.05$ , data not shown).

## DISCUSSION

Initial animal dirtiness score assessment was conducted as cows were assigned to winter accommodation treatment, and so were typical of dirtiness scores of cows at pasture (all animals were managed at pasture prior to the experiment). In this experiment the dirtiness scores of animals that were sheltered from the weather did not change significantly from the initial inspection for the majority of the accommodation period, regardless of whether they were indoors in cubicles, or outdoors on a sheltered OWP. In contrast, animals on both unsheltered OWPs had higher dirtiness scores than at the beginning of the trial at all but the last inspection, which occurred after the OWP's were cleaned. In comparison to the unsheltered pad where cows were fed from a concrete feed face that was cleaned regularly, the feeding area of the second unsheltered OWP with the self-feed silage was much dirtier as it was not possible to remove soiled material on a regular basis in this system. Nevertheless, there was no difference in animal dirtiness scores between both unsheltered pad designs. This is probably due to cows in the self-feed system selecting areas away from the feed face area to lie down. Although previous work found no effect of shelter on animal hygiene (Hickey et al., 2002) cattle in that experiment were only sheltered by windbreaks and not overhead from rain. Thus moisture is an important factor determining animal cleanliness probably because moist faecal matter attaches to an animals coat more easily than dry matter.

In a similar experiment (O'Driscoll et al., 2006) animals that were accommodated on a sheltered OWP at a high stocking density ( $6\text{m}^2$  woodchip area/head), had much higher dirtiness scores than animals accommodated on an unsheltered OWP at a lower stocking density. This suggests that a high stocking density in sheltered OWPs negates any positive effects of shelter on animal cleanliness. One reason why animals at the high stocking density had high dirt scores is that a high stocking density not only increases the number of animals per area woodchip, but also results in a higher manure load on the woodchip area. Although in a sheltered OWP manure may not be as moist as on an uncovered OWP, a greater volume of manure may result in a thick fecal layer building up more quickly, and probably more contact between the animals' coats and manure when they lie down. Findings from this study, however, clearly demonstrate that overhead shelter in itself does not result in high animal dirtiness scores

Furthermore, O'Driscoll et al. (2006) reported that the combined incidence of clinical and sub clinical mastitis was higher in the sheltered OWP than in either the unsheltered OWP or indoors in cubicles ( $P < 0.05$ ). Infectious agents were also isolated in that experiment from more animals in the sheltered OWP than in the other two treatments three weeks post calving. Intra-mammary environmental pathogens are significantly associated with udder hygiene scores (Schreiner and Ruegg, 2003) so it is likely that dirty conditions led to these udder health problems. The lower stocking density in the sheltered OWP in this experiment resulted in superior animal hygiene, and this is reflected in the lack of difference in incidence of mastitis, sub-clinical mastitis, and the presence of intra mammary pathogens between treatments.

After cleaning of the OWPs and application of fresh woodchip, animal dirtiness scores on the uncovered OWPs returned to a level similar to that recorded indoors in cubicles and in the sheltered OWP. This may have important management applications. Assessment of animal

hygiene by the stockperson during the dry period, and subsequent removal of dirty animals to clean accommodation, or replacement of bedding, may improve animal hygiene during the dry period. There is evidence that intra-mammary infections that occur during the dry period can cause clinical disease post-calving (Green et al., 2002), and thus these management practices may reduce the risk of developing intra mammary infection during the following lactation.

Results from this study indicate that dairy cow hygiene is affected by the cleanliness of bedding, and also by the presence of shelter from weather. However, although animals on both uncovered OWPs had higher dirtiness scores than animals in the other accommodations, this did not affect SCS or mastitis incidence post-calving. Therefore management of the OWP's was sufficient so that milk quality and animal health was not compromised when compared to animals accommodated in traditional indoor cubicles during their dry period. These findings have important implications for the management of dry cows.

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## INFLUENCE OF DIGITAL DERMATITIS AND SOLE ULCER ON DAIRY COW BEHAVIOUR AND MILK COMPOSITION

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**Keywords:** dairy cow, digital dermatitis, sole ulcer, milk production, behaviour

### ABSTRACT

The aim of this study was to investigate whether digital dermatitis (DD) and sole ulcer (SU) in high producing dairy cows were associated with behavioural changes, other health problems, milk production and milk composition. The study was carried out on a research dairy farm with about 300 milking cows of Swedish Red and Swedish Holstein breed. Lactating cows were kept in a cubicle system with scraped alleys and were mostly in their first and second lactation. Clinical examination of the claws of all cows was made at claw trimming. Cows scored for mild or medium degree of DD or SU were listed, and ten cows suffering from each claw disorder were selected for the study. For each diseased cow a paired healthy control cow was selected blocked on breed, age, parity and lactation stage. General clinical examination of each cow used in the study was performed before the first observation period started. A second clinical examination of the claws was performed between the second and third observation period. Behavioural observations were made on paired cows (one with DD or SU and one healthy cow) during four periods with the 0–1 sampling method. Control milking results were collected monthly from February to April and milk production records once per six days. For statistical analyses Generalised Linear Models (Proc GENMOD) in SAS Version 9.1 were used.

The study showed that cows with DD were lying significantly less than healthy cows during period one ( $p < 0.05$ ) but not during the other periods. Cows with SU were lying down significantly less than healthy cows during the first period ( $p < 0.01$ ). Cows with SU walked significantly more than healthy cows during period one ( $p < 0.05$ ) and they walked significantly less than healthy cows during period three ( $p < 0.001$ ). Healthy cows stood and ruminated significantly less during period one than cows with SU ( $p < 0.05$ ) and DD ( $p < 0.05$ ). During the second period healthy cows had a tendency to stand and ruminate less than cows with DD ( $p = 0.0731$ ).

During the study cows with DD sent significantly less ( $p < 0.05$ ) and cows with SU received significantly less social licking than healthy cows ( $p < 0.01$ ). During the study cows with SU sent significantly less butting and pushing than healthy cows ( $p < 0.01$ ) and also received significantly less butting and pushing ( $p < 0.01$ ).

In period one and two cows with DD produced significantly less kg of energy corrected milk (ECM) than healthy cows ( $p < 0.05$ ). They had a tendency to produce less ECM than healthy cows during the fourth period ( $p = 0.0731$ ). Cows with SU had a tendency to produce less ECM than

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healthy cows during the second period ( $P=0.0561$ ). Cows with SU had a tendency to have higher milk fat percentage during the third period ( $p=0.0954$ ). In period two and three cows with SU had significantly higher somatic cell count than healthy cows ( $p<0.05$ ,  $p<0.01$ ) and cows with DD had a tendency to have lower cell count than healthy cows in period four ( $p=0.0543$ ).

It was concluded that DD and SU have an influence on behaviour and milk yield in high producing dairy cows. The study emphasizes the benefit of early detection and treatment of claw diseases as well as the importance of prevention measures in order to minimize the influence on behaviour and production.

## THE VIRTUAL FARM

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### SUMMARY

The Virtual Farm is an internet based computer program designed to create 3D-models of farms with animals in it. It will be the outcome of a big cooperative effort, lasting for several years. The completion of this ambitious project requires combining existing knowledge and future research.

The key Finnish players in the Virtual Farm cooperation are Savonia University of Applied Sciences, University of Kuopio, University of Helsinki, FarmiMalli Ltd., and some private companies. Other domestic and foreign universities and companies are welcomed to participate in further development of the idea. This paper includes the outline of the Virtual Farm idea.

**Keywords:** virtual farm, three dimensional, ethogram, computers

### THREE STEPS TO THE VIRTUAL FARM

#### 1. Drawing the farm with the Virtual Farm Designer

The Farm Designer is a computer program ([www.farmdesigner.com](http://www.farmdesigner.com)), which can be accessed easily with any internet browser (Figure 1). It is possible to design a farm building by simply dragging the walls, windows, doors and other facilities to the desired place with a mouse. The results can be seen immediately three-dimensionally. There is a possibility of “flying” inside and outside the building and looking closely at details. The program has been developed by FarmiMalli Ltd.

In the latest version of the Farm Designer, the same drawing can be manipulated from different locations simultaneously. This allows for the the farmer and building designer to plan the farm together on the internet, seeing the drawing in three dimensions, and being able to move walls and facilities to their desired places.

A demo of the program showing a barn of 50 cows (without the actual animals) is available on the internet (<http://www.annemanni.fi/index.php?id=442>). After installing the program “Barn.exe”, it is possible to move both inside and outside the barn by following the instructions on the screen.

#### 2. Modelling virtual animals

The second step is to model virtual cows and other animals. To model animals information about how the animals look and move, their daily activities, their behavioural patterns and their location is needed.

Animating an animal starts by creating a skeleton with all joints in place. Muscles and skin can then be added to the skeleton.

Cows and other animals can be made to look very realistic by using special animation techniques, but the difficulty is to make them move like real animals. This is achieved by using a combination of an ethogram – a list of descriptions of the behavioural patterns of a species – and animation. This process has been started at University of Kuopio, Finland with behavioural studies done to compile an ethogram of cattle. With the aid of a literature review and observations of animals, almost a hundred different behavioural patterns of cattle were titled, described, photographed and will further be video-recorded. Using the video ethogram, the animated cows can be made to move and perform different behaviours accurately and as lifelike as possible.

Information of the location of cows in a barn will come from a project called “Very Intelligent Cow Barn” run by University of Kuopio. In the project, the exact location of all cows in a real barn is received every second. Location of the real cows will be transferred to be the location of the animated animals. Now, with the help of the cattle ethogram and the location information it is possible to make the virtual cows move correctly in the right places.

The 3D model of virtual animals will help tremendously in the functional design process of new farm buildings. The coming building with cows (or pigs, horses, sheep etc.) moving in it can be visualised before anything has been built.

### **3. Simulation**

Simulation, the third and final step of the process, is the most challenging. This is where international cooperation is needed. The aim is to simulate different occurrences (fire, mastitis epidemics, hoof trimming, fertility problems, etc.) in the virtual farm. It is theoretically possible to create an endless amount of simulations; if a way to make the virtual animals react to them in a certain way can be found.

There are still plenty of uncertainties about the simulation process, for which there are currently no solutions. The goal is to create “intelligent” virtual animals, so that information of research work could be included into the behavioural patterns of the virtual animals.

Interested research groups and enterprises are warmly welcomed to discuss with the authors about possible cooperation at this stage.



**Figure 1.** The Farm Designer is an internet based program designed to create 3D models of farms. It is possible to move freely inside and outside of the building and look at details. The 3D farm is the first step towards the Virtual Farm Project. Once finished, it will feature virtual animals that look and move in an authentic manner and react to the changes in the environment.

## LINKS

The Farm Designer, <http://www.farmdesigner.com>

The Farm Designer Demo, <http://www.annemanni.fi/index.php?id=442>

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## MICROCLIMATE IN COWSHEDS IN FINLAND AND ESTONIA

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### SUMMARY

A series of microclimate measurements were performed in different kinds of cow houses in Estonia and Finland. The number of animals in the structures varied from 30 to 600. Measurements were made in summer and winter conditions with ambient temperatures from –30°C to +30°C. The results showed that there were differences in microclimate depending on design of structures, outside temperature, wind and ventilation rates. The recommended values for microclimate in the cow structures were mainly within the recommendations.

**Keywords:** microclimate, ventilation, cold, uninsulated, semi-insulated barns, cow houses

### INTRODUCTION

Due to the lower investment, capital and construction costs, cold un-insulated and semi-insulated cow structures have been of interest in recent times. The building cost for the framework and walls are estimated to be about 15% lower in semi-insulated and 35% lower in non-insulated cubicles than in fully-insulated free stalls structures (Jeppsson et al., 2006). In the last 15 years about 310 semi-insulated structures have been built in Finland consisting of about 15 to 40 animal units (Brännäs, 2005). Presently, Estonia has over 60 large semi-insulated structures housing between 300–1000 animal units each (Kivinen et al., 2006), and about 90 new or renovated uninsulated cowshed between 2002 and 2006 (Pajumägi, 2007).

**Table 1.** Nationally acceptable concentrations in dairy animal structures (MMM) and the harmful concentrations limits to humans in Finland (MSAH, 2005)

Gases	Limits in animal structures (ppm)	Exposure limits to humans	
		(ppm)	(mg/ m <sup>3</sup> )
Carbon dioxide, CO <sub>2</sub>	3 000	5000 (8 hrs)	9100 (8 hrs)
Ammonia, NH <sub>3</sub>	10	20 (8 hrs), 50 (15 mins)	14 (8 hrs), 36 (15 mins)
Hydrogen sulphide, H <sub>2</sub> S	0,5	10 (8 hrs), 15 (15 mins)	14 (8 hrs), 21 (15 mins)
Carbon monoxide, CO	5	30 (8 hrs), 75 (15 mins)	35 (8 hrs), 87 (15 mins)
Organic dust	10 mg/m <sup>3</sup>	–	5 (8 hrs), 10 (15 mins)

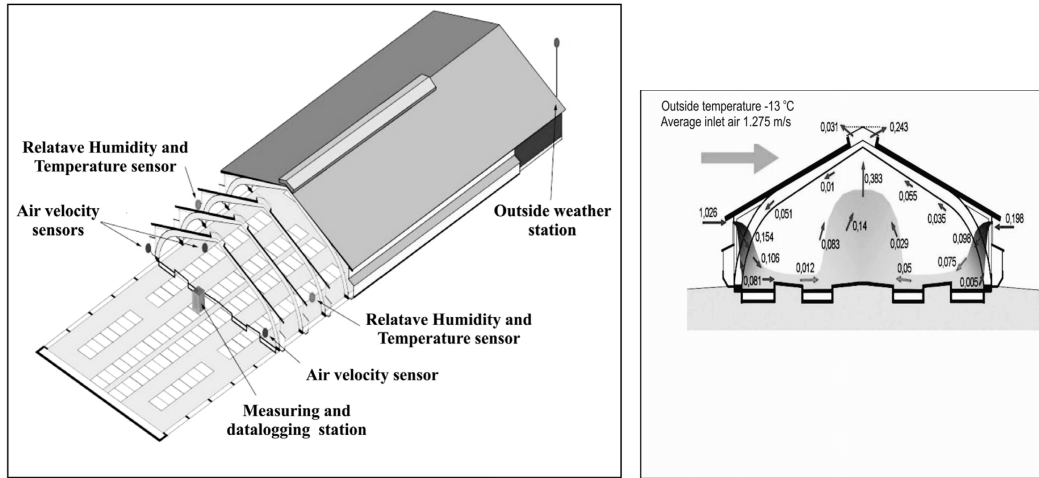
Estonia and Finland experience weather conditions ranging from –35 °C to +35 °C. This varying weather prevailing in the winter and in the summer makes it difficult to ensure suitable diurnal

microclimatic conditions for the animals in dairy buildings. Poor microclimate in animal structures and high gaseous concentrations can increase the occurrence and severity of certain endemic diseases (Amon et al., 2001). Several authors have shown that gaseous concentrations are often too high in animal structures (Zhang et al. 2005). The European Directive 2001/81/EC on National Emission Ceilings, sets upper limits for the total amount of emissions from each Member State for the total emissions of gases like Sulphur Dioxide (SO<sub>2</sub>), Nitrogen Oxides (NO<sub>x</sub>), Volatile Organic Compounds (VOCs) and Ammonia (NH<sub>3</sub>). However, the directive leaves it largely to the Member States to decide which measures to take in order to comply. In Finland the building regulation of the Ministry of Agriculture and Forestry (MMM-RMO C2.2, Table 1) specifies some recommended microclimatic conditions in livestock structures. In addition, the Ministry of Social Affairs and Health in Finland (MSAH 2005, Table 1) and Labour Inspectorate Estonia under the Ministry of Social Affairs have exposure limits for indoor air conditions for workers. In Estonia some guidelines for cow protection in cow structures can be found (RTL, 124, 179. 2002). For workers, indoor microclimatic standards like EVS 839:2003 that deal with indoor air quality for humans and EVS 845:2004 for ventilation are also available. Typically, where recommendations are unavailable, animal structure designers try to go according to the recommendations given by the CIGR commission. For relative humidity (RH) in animal structures, CIGR (1984) recommends maximum and minimum values as a function of indoor temperature, for example, a RH of 50–90% at 0°C followed by a steady decrease of RH to a tolerable range of 40–60% at 30°C. In Finland, the MMM-RMO C2.2 recommends an optimum RH of 50 to 80% and optimum temperature conditions for dairy cows to be between 5–15°C. Lower and upper critical temperatures were proposed to be –15°C to –25°C and 23 to 27°C respectively.

The objective of this research paper was to find out the microclimate conditions of different types of cow structures during varying climate conditions. It will also assess whether these microclimatic conditions meet national recommendations.

## MATERIALS AND METHODS

A series of microclimate measurements were performed in different kinds of cow structures in Estonia and Finland. The number of animals in the structures varied from 30 to 600. The measurements included both summer and winter conditions and the ambient temperatures from –30°C to 30°C. The buildings included uninsulated and insulated structures. Three different types of measurement systems were used. A stationary multiple-sensor measuring station (Fig. 1) and wireless measurement system, both for long period measurements, and a mobile multiple-sensor measurement system for short period measurements. Typical sensor locations of a stationary measurement system are as shown in Fig. 1. A set of temperature, radiative heat, heat flux, relative humidity, ammonia, carbon dioxide, hydrogen sulphide and air velocity sensors were placed at 0.5, 1 and 1.5 m heights inside the measuring and data logging station. The stationary measurements were completed with more precise and periodical gas and ventilation measurements. Gas measurements were performed with a Fourier Transform Infrared Spectrometry (FTIR) multi gas analyzer. Measurement of air velocity was done using multiple hot-wire and 3-dimensional ultrasonic anemometers (Fig. 1). These measurements were done in one day and continuous measurements in 1–4 months.



**Figure 1.** Measurement set-up and location of sensors for microclimate studies (left). Velocity profile in cowshed (right).

Carbon dioxide balances were employed in the estimation of emissions. The calculations were made based on the conservation of mass and energy in the building, under steady-state conditions. The ventilation flow through the animal structure,  $q_v$  in  $\text{m}^3 \text{h}^{-1}$  the gaseous emissions  $E_g$  were estimated according to Eq. (1), where  $C_{prod}$  is the production of  $\text{CO}_2$  in  $\text{m}^3 \text{h}^{-1}$ ,  $C_{in}$  and  $C_{out}$  is the  $\text{CO}_2$  concentrations in the indoor and outdoor air in ppm.  $\Delta C_g$  is the difference between the inside and outside gaseous concentrations of the individual gases in ppm.

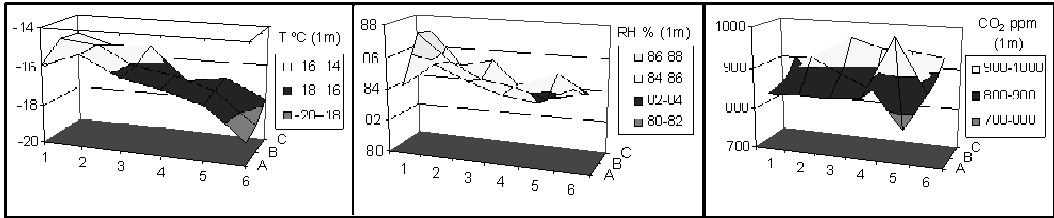
$$q_v = \frac{C_{prod}}{C_{in} - C_{out}} \quad (1)$$

$$E_g = q_v \Delta C_g$$

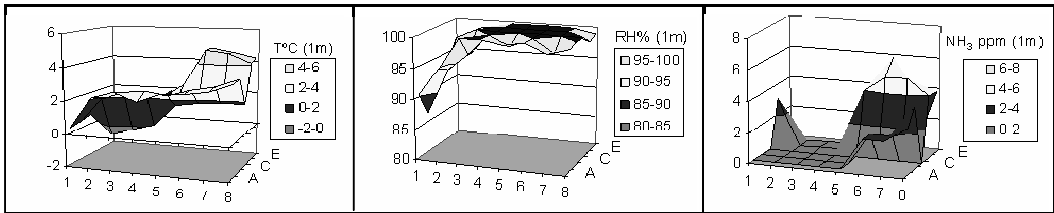
## RESULTS AND DISCUSSION

The intermittent and continuous measurements provided information about typical gas concentrations and microclimates in dairy structures under moderate to extreme winter and summer conditions. All the cow structures except F5 and F6 had natural ventilation (table 2). The differences in cowshed structural designs and manure handling methods contributed to the ventilation and microclimate. Ventilations rates were very variable and airflow velocities were between 0.1 and 0.7 m/s at 1m in the centre of the cow structures (table 2).





**Figure 2.** Spatial variation in microclimatic conditions in dairy barn (winter, Finland)



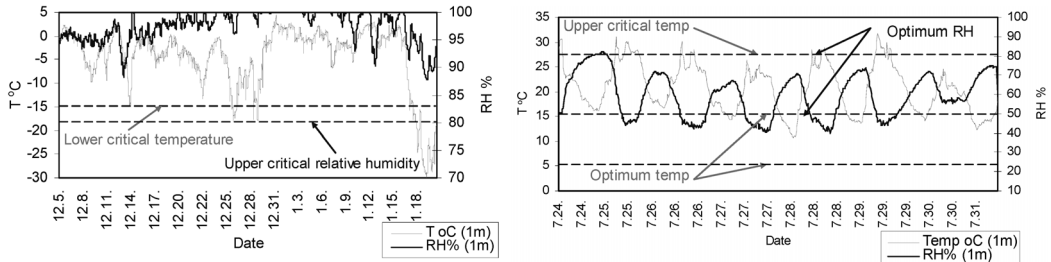
**Figure 3.** Spatial variation in microclimatic conditions in dairy barn (winter, Estonia)

**Table 2.** Microclimate in cow structures in Finland and Estonia. Notation: the place coding is as follows E=Estonia, F=Finland, 1–7=site number, W=winter, S=summer. V is ventilation; Vol is volume of cow structure.

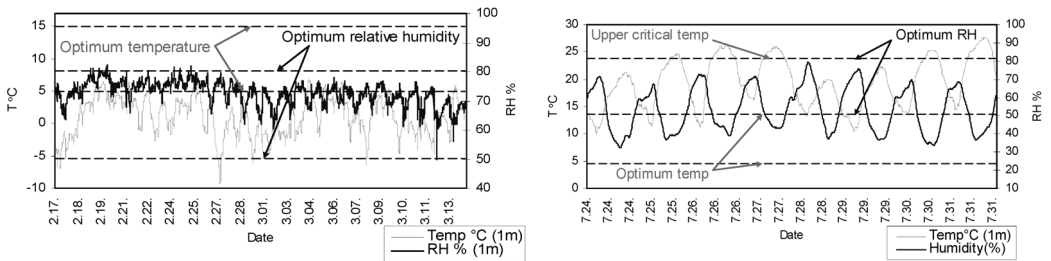
Place	Cowshed type	Number of Cows	Vol (m <sup>3</sup> ) × 10 <sup>4</sup>	T <sub>in</sub> (°C)	T <sub>out</sub> (°C)	v <sub>in</sub> (m/s)	v <sub>out</sub> (m/s)	RH <sub>in</sub> (%)	RH <sub>out</sub> (%)	CO <sub>2in</sub> (ppm)	NH <sub>3in</sub> (ppm)	CH <sub>4in</sub> (ppm)	V (m <sup>3</sup> /h) × 10 <sup>3</sup>
E1W	semi-insulated	480	45	-1	-2	0.2	2.3	91	74	522	1.1	17	616
E1S		460	45	28	27	0.6	2.5	39	38	454	1.6	8	700
E2W	semi-insulated	500	66	1	-3	0.1	1.7	89	72	775	1.6	34	233
E2S		500	66	27	28	0.3	0.1	46	40	751	9.7	40	262
E3W	semi-insulated	500	53	2	0	0.1	0.4	87	90	1171	3.3	64	116
E3S		500	53	29	30	0.3	0.4	44	37	375	3.0	9	–
E4W	semi-insulated	600	64	0	-4	0.1	4.2	86	85	854	3.2	40	233
E4S		600	64	28	29	0.6	2.5	47	47	412	17.0	14	783
E5S	semi-insulated	500	53	30	32	0.7	1.0	38	28	397	2.6	11	2865
F1W	uninsulated	55	24	-17	11	0.1	3.8	84	83	856	0.2	39	20
F2W	semi-insulated	50	25	1	-1	0.3	3.2	82	68	1225	15.4	42	11
F3W	semi-insulated	95	10	4	-12	0.1	1.4	84	85	1678	7.0	116	13
F4W	semi-insulated	80	11	3	-12	0.1	1.6	84	85	1678	3.5	58	11
F5W	fully-insulated	70	5	8	-8	0.1	0.1	73	74	1622	3.4	110	10
F6W	fully-insulated	60	10	12	1	0.3	0.7	75	80	1545	8.0	86	9
F6S		60	10	19	18	1.0	0.2	54	47	757	2.6	20	26
F7W	uninsulated	50	6	6	3	0.1	2.9	91	86	550	1.3	17	55
F7S	uninsulated	50	6	29	26	0.2	2.7	45	41	634	4.7	18	35

There were high spatial variability in microclimatic conditions recorded in both Finnish and Estonian cow structures. Fig. 4 and 5 show the typical diurnal variability. The results showed that there are diurnal differences in microclimate depending on outside temperature, wind and ventilation rates (Table 2, and Fig. 4 and 5). The ventilation rate was mainly affected by the ventilation opening sizes of the buildings (natural ventilation) which in turn contributed to the microclimate.

The recommended values for microclimate in cow structures were exceeded when the ventilation was inadequate (Table 2, and Fig. 4 and 5). The most eminent problems were related to high moisture content (RH) and freezing of moisture or water during cold weather (Fig. 4 and 5). In some cases, temperatures in the uninsulated cow structures were below the lower critical temperatures (Fig. 4 and 5). In the summer period, there were days when the recorded temperature went above the upper critical temperatures (Fig. 4 and 5). Carbon dioxide concentrations were in the range of the recommended levels in all cases. In some cases, methane concentrations were more than 10 times the recommended levels (table 2). Ammonia emissions were mostly below 10 ppm-vol in both Finnish and Estonian cow structures.



**Figure 4.** Winter (left) and summer (right) microclimatic conditions in a cow structure in Finland



**Figure 5.** Winter (left) and summer (right) microclimatic conditions in a cow structure in Estonia

## CONCLUSIONS

With proper ventilation rate the microclimate can be kept within recommended values. Winter conditions present especially moisture problems and freezing of moisture, water and manure. Normally there was only one or two measured gas or value, which was outside the recommendation.

The basis of the microclimate recommendations were difficult explain, they seemed to be derived mainly from human exposure limits.

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## INFLUENCE OF MILK FEEDING METHODS ON THE WELFARE OF DAIRY CALVES

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### SUMMARY

Problems occur in raising dairy calves during the milk feeding period due to the occurrence of abnormal behaviours and health problems. Offering calves an artificial teat to suck the milk from, with a low flow of milk and access to the teat at all times reduces cross-sucking between calves. In computer controlled milk feeding systems calves may perform cross-sucking and often have reduced health. Calves suckling the dam restrictively or ad libitum or foster cows show no signs of cross-sucking. Calves can be raised with a good welfare if they are housed and milk fed according to their behavioural needs.

**Keywords:** cattle, dairy calves, suckling, milk feeding, behaviour, cross-sucking

### OBJECTIVE OF REVIEW

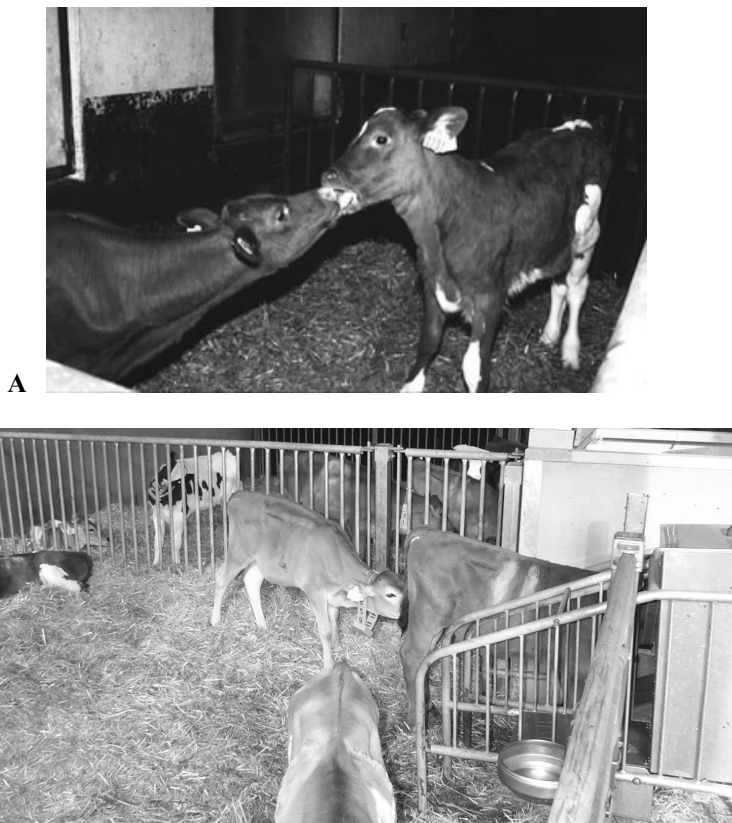
The objective of this paper is to highlight problems with current milk feeding methods to dairy calves, and to suggest developments of the milk feeding system to dairy calves in order to avoid abnormal behaviours and health problems. This will be done by presenting research results and developmental work done primarily in Sweden in collaboration with Denmark, Norway, Finland and Mexico.

### THE PROBLEM

Dairy calves are in most countries removed from the dam shortly after birth and raised in single or group pens during the milk period. There are many different ways of feeding milk to calves, but one common method is to provide restricted amounts of whole milk or milk substitute in open buckets or troughs to the calves at two meals per day. They usually receive a restricted amount per calf of 2–3 l./meal. The milk is usually finished within one minute and thereafter calves start sucking on different parts of the body of each other, so called cross-sucking (Fig. 1a), or they suck on the bucket or other fittings of the pen (Lidfors, 1993). This abnormal sucking is most intense during the first six minutes, where after it declines until 15 minutes after the milk meal when it has almost stopped (Lidfors, 1993; Lidfors, 1994). If calves are kept in single pens they can only reach to suck on the mouths and ears of neighbouring calves. If they are raised in group pens they can also suck under the belly of pen mates. When they suck on the navel of others it can lead to oedema and navel infection. Dybkjaer (1988) reported that hair loss and inflammation of sucked body parts can occur.

When heifer calves are being sucked under the belly it may be the undeveloped teats that are sucked. This behaviour is called intersucking, and has been defined by one animal touching the udder region of a group member with its mouth and trying to get hold of the teat with the intention of sucking milk (Keil and Langhans, 2001). Milk sucking, milk theft or galactophagia are other synonyms for intersucking, which are usually used for cows that also succeed in swallowing milk from the teat of another cow (Lidfors and Isberg, 2003). In a questionnaire answered by telephone interview of 230 Swedish farmers there was a significant relationship between calves sucking under the belly of other calves and heifers intersucking ( $P < 0.001$ ), heifers intersucking and cows intersucking ( $p < 0.05$ ) and cows intersucking and cows sucking on themselves ( $p < 0.01$ ) (Lidfors and Isberg, 2003).

Intersucking in heifers may be a risk factor in relation to teat injuries and secretion of a milky substance (Von Burmeister et al., 1981), and farmers have reported heifers to start producing milk as a response of intense intersucking. Some researchers have pointed out that if calves are fed milk from cows with mastitis and if they then suck on the teats of other heifer calves there is a risk that the bacteria is stored in the teats and udder until the day the heifer starts producing milk (Barto et al., 1982; Robertson et al., 1990).



**Figure 1.** Cross-sucking calves when housed in pairs (A: photo Jenny Loberg), and computer controlled milk feeding system to dairy calves (B: photo Per Peetz Nielsen)

## RAISING CALVES ON THE DAM OR FOSTER COWS

Calves may be left with their mother, and on organic farms in Sweden there is a rule stating that calves should be left with the dam for at least the first four days (KRAV-rules, 2006). Some farmers find ways of letting the calves suck for longer times, and then it is often done by letting the calves suck twice or three times per day, in a restricted suckling method (Hartman, 1994, Anderberg, 2001). Restricted suckling is often done in countries where *Bos indicus* or crosses between *Bos taurus* and *Bos indicus* cows are milked, because the cow releases the milk better if she has her calf next to her, and the calf is allowed to suckle the residual milk which would otherwise be left in the udder. This milk may also be valuable for the calf as it is higher in fat percentage and gives the calf a higher MJ (Gratte, 2005).

One of the problems with restricted suckling is that if calves are allowed to suckle directly after milking some dairy cows keep their milk in the udder during milking and then release it to the calf when it suckle (Hartman, 1994; Fröberg et al., 2005). However, the calf is not able to drink all the milk in the udder, and thus leave some teats un-suckled (Jung, 1994; Gratte, 2005). In order to get the cows to release the milk farmers may then have to give the cow an injection with oxytocin, which may cause negative effects on milk let-down if used too frequently.

Alternatively, dairy calves may be raised by a foster cow, i.e. a cow which nurses alien calves. A study showed that most dairy cows of the Swedish Holstein and Swedish Red breed accepted four alien calves when they were presented to them at four different times during the lactation (Loberg and Lidfors, 2001). However, accepting to be nursed is not the same as adopting the calves as if they were her own calves, and a study on this showed that some cows adopts all calves whereas more cows adopt only one or two of the alien calves (Nielsen et al., 2007). At weaning and separation from the foster calves the foster cows may react negatively and vocalise, try to get back to the calves (Loberg et al., 2007a), and not releasing milk to the milk machine (Hernandez, 2005). The use of a weaning plate on the muzzle of the calves so that they were weaned from milk before they were separated from the foster cow lead to a significantly smaller reaction at separation both in the foster cow and her calves (Loberg et al., 2007a, b).

## RAISING CALVES ON COMPUTER CONTROLLED MILK FEEDERS

New animal welfare regulations and increased herd size within the Nordic countries has lead to that a larger number of calves are housed in groups today. Within EU calves above 8 weeks must be group housed (Council Directive 97/2/EC, 1997), and on organic farms calves must be group housed already from 1 week of age (Council Regulation 1804/1999/EC, 1999). Group housing of young calves has beneficial effects on their development of movements, play and social skills (Jensen et al., 1999). In order to facilitate milk feeding to calves in large groups and to let them suck in connection to milk intake computer controlled milk feeding systems have been developed by different companies (Fig. 1b). However, there are some problems with this system. First of all it is usually based on having 20–30 calves in the same pen, and if the farm is small calves usually varies in age thus making it impossible to have an “all-in-all-out system” where pens can be cleaned between groups of calves.

Grouping of unfamiliar cattle has been found to increase aggression, social stress, locomotion behaviour and to have negative effects on feed intake and milk yield (reviewed by Bøe and Faerevik, 2003). An investigation of the social preferences of calves showed that pre-weaned calves established social preference already after 3 weeks of grouping, and that calves separated

during a Y-maze test spent more time with a familiar calf (Færevik et al., 2006). During a separation test calves showed fewer sign of separation stress when separated together with a familiar calf compared to with an unfamiliar calf, or alone (Færevik et al., 2006).

In large groups there are larger risks of getting health problems. In a study on 136 dairy farms with a total number of 3081 calves in Sweden it was found that cases of diarrhoea were significantly more severe in calves housed in large group pens (more than 10 calves) than in individually housed calves (Svensson et al., 2003). There was a tendency that cases of diarrhoea was more severe in calves housed in large groups than in calves housed in small groups (less than 10 calves) (Svensson et al., 2003). In a recent study it was found that diseased calves were drinking as much milk as healthy calves, but that they reduced the number of times they visited the automatic milk feeders to check if they would get milk, so called unrewarded visits (Svensson and Jensen, 2007). The research project will continue and aims at finding reliable ways of using the data that comes out from the computer controlled milk feeding system to detect sick calves at an early stage.

Another problem with this system is that calves must drink milk one by one, and the synchronised behaviour typical of cattle can not be performed. A number of studies have been done to study the effects of number of calves per feeder and number of milk portions on use of the feeder and social behaviour (Jensen, 2004), on the effect of milk allowance and weaning type (Jensen, 2006), on the effects of milk feeding method and group size on feeding behaviour and cross-sucking (Jensen and Budde, 2006), and on how the age at introduction to the group affects dairy calves' use of a computer-controlled milk feeder (Jensen, 2007). In a recently published study we found that calves that got a high milk allowance (9,2 L./day) used less time in the milk feeder than calves that got a low milk allowance (4,8 L./day), because they had fewer visits where they were not given any milk (Nielsen et al., 2007b). A gradual weaning of milk during 14 days lead to a reduced number of visits without getting milk, and a larger intake of concentrate during the first week after weaning compared to calves abruptly weaned (Nielsen et al., 2007). It was also found that the abruptly weaned calves performed more cross-sucking both during and after weaning than the gradually weaned calves (Nielsen et al., 2007).

The flow of milk in the teats of the computer controlled milk feeders were reduced in one study in combination with a large or small meal. The data is currently under evaluation, but the hypothesis is that if calves can drink the milk slower and get a larger meal size they would not cross-suck as much on each other and they would not visit the milk feeder so frequently to check if they will get milk again.

### **RAISING CALVES ON ARTIFICIAL TEATS IN SMALL GROUPS**

In a study where the milk was delivered slowly into an open bucket during 10 minutes the reduced milk intake lead to that calves spent the entire time licking the milk and they performed less cross-suck during and after milk intake (Loberg and Lidfors, 2001). When the same calves were allowed to suck on a teat floating in the milk of the open bucket they also cross-sucked less (Loberg and Lidfors, 2001). The combination of low milk flow and the possibility to suck gave the lowest amount of cross-sucking (Loberg and Lidfors, 2001). When calves are fed milk in teat-buckets cross-sucking may be reduced, but if the teat-buckets are removed immediately after the milk is finished they start cross-sucking (Jung and Lidfors, 2001). Putting up barriers between calves during milk intake is an effective way of reducing cross-sucking (Jensen et al., 2007). The

larger amount of milk calves are fed at a meal the less cross-sucking is performed after finishing the milk (Jung and Lidfors, 2001).

### THE FUTURE

In order to reduce cross-sucking calves should be able to suck the milk, have large enough meals that cause satiety and having ad libitum access to concentrate, silage and hay so that they have something to chew on if hungry. This also leads to a development of their rumen function. In small groups calves can more easily be kept with a small age difference, which reduces competition and health problems.

### CONCLUSIONS

It is concluded that calves can be raised with a good welfare if they are housed and milk fed according to their behavioural needs.

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## POSTER PRESENTATIONS

### USING COPPER SULPHATE, PERACETIC ACID OR A COMBINATION OF BOTH IN FOOT BATH FOR THE CONTROL OF DIGITAL DERMATITIS AND HEEL HORN EROSION IN DAIRY COWS

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#### ABSTRACT

Footbath for dairy cows should be a complement but not a substitute to optimal hygienic precautions in the management system. The aim was to test the effectiveness of different methods and different disinfectants in a dairy herd with serious infectious claw diseases.

A foot-bath with two longitudinal Hoofmat™ equipped compartments was used in two experiments. Firstly, 7% copper sulphate solution (left) and 15 litres of water (right) was used twice daily for 112 cows after milking. Secondly a combination of both copper sulphate and peracetic acid in water solution (DeLaval) was similarly tested on 240 cows. In a third experiment 101 cows walked twice daily through a passage where foam containing peracetic acid and hydrogen peroxide (Kovex®, Ecolab) was spread, while 66 cows were used as untreated controls. All infectious related claw lesions were recorded at the beginning and at the end of the study, or when cows were leaving or entering the milking herd during the up to four months observation-period. The analysis was performed at the foot level, adjusting for cow-level clustering.

Almost all cows were affected by heel-horn erosion, digital dermatitis were seen in every fifth cow and interdigital hyperplasia in every tenth cow at the start of the study. Foot bath with pure copper sulphate had a positive effect on all studied traits, decreasing the odds of having heel-horn erosion and either heel-horn erosion or digital dermatitis at the end of the study period by 4 times, and decreasing the odds of having digital dermatitis by 10 times, increasing the odds of improvement of heel-horn erosion and either heel-horn erosion or digital dermatitis by approx. 2.5 times, and the odds of no deterioration of the same type of lesions by between 6 and 7 times. Using the combination of Copper sulphate and peracetic acid gave a reduction of heel horn erosion to half but had no effect on digital dermatitis. The study provides no evidence of an effect of a foam bath containing peracetic acid and hydrogen peroxide during 56–113 days of exposure on heel-horn erosion, digital dermatitis or interdigital hyperplasia. It is likely that the effect of copper sulphate is dose dependant. However, copper sulphate is though disputable as being a heavy metal with environmental accumulation and the disposal should be restricted to copper deficient soils. It is urgent to find alternative and effective disinfectants to antibiotics and otherwise undesirable products.

## ABOUT DEVELOPMENT OF THE MODEL OF INFLUENCE OF BIOLOGICALLY ACTIVE SUBSTANCES ON THE RESISTANCE AND EFFICIENCY OF BULLS

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### SUMMARY

The influence of biologically active substances (BAS) on the resistance and efficiency of bulls of black and white breed in different age groups has been researched. It has been used the vegetable origin preparations and substances that were formed as a result of microwave hyperemia of bulls testicles. It has been stated that the efficiency of the use of biologically active preparations depends on the age of animal and the level of its body.

The model of influence of BAS has been developed and incomplete square-law equation of regression has been offered. The hypothesis on the formation of tissue auto stimulators under the influence of microwave hyperemia of testicles has been confirmed. The suggested methods of interior parameters in bulls provide the increasing in resistance and efficiency at 10–15%.

**Keywords:** biologically active substances, resistance, microwave, testicle, hyperemia, bulls, model regression

### INTRODUCTION

BAS are widely used in practice of animal industries. They allow to reduce negative influence of infringements of conditions of the maintenance and feeding on growth and development of animals [1]. The efficiency and resistance of animals can be increased by their help. BAS very often have a wholesome effect on the offspring [2, 3]. The importance of their role in the removing process of radionucleids from animal and human body [4] can scarcely be exaggerated.

Practically in all scientific works of this tendency the effect mechanism of BAS is examined and the optimal preparation doses is determined, but as a rule the investigations are carried out on animals of only one definite sex age group and during one or several stages of body development.

The complication of effect mechanism of BAS on the animal body is mentioned in all scientific works.

### OBJECTIVE

To study the influence of BAS on the resistance and efficiency of bulls of black and white breed the research was carried out on animals, aged 6–8 months.

Fitostimulators “Agroperon” (an extract of wheat grass creeping), “Humosvit” (made on the basis of ecologically pure land and sea flora) and tissue auto stimulators produced under the effect of microwave hyperemia of bull testicles have been used in the experiment.

This scientific work has been directed to study the peculiarity of the effect mechanism of BAS in animals of different age.

## MATERIALS AND METHODS

To carry out the investigation the animals were divided into two groups: group 1 – bulls of 6 months age, group 2 – bulls of 8 month age. Inside each of the above mentioned groups there were four subgroups that were formed according to the scheme of complete two-factor experiment. Bulls of control subgroups were not given any treatment. They were used and considered in all three experiments and due to that the number of subgroups was reduced to eight.

This experiment lasted 170 days (the preparatory period lasted 14 days and the basic period – 156). The estimation of productivity was made by calculations of an absolute daily average gain weight. Natural resistance was determined by the account humoral and cellular factors of protection.

The investigations according to the control of hypothesis about formation of tissue auto stimulators under the influence of microwave hyperemia of testicles in bulls [5] were marked out in the separate group. For testicles heating the experimental setup NG-1 developed at the A. Ya. Usikov Institute of Radiophysics and Electronics of National Academy of Sciences of Ukraine (Kharkov, Ukraine) were used.

This setup consisted of the source of microwave irradiation at frequency of 2,45 GHz and the microstriped-microslitted applicator. Exposition time was determined by the timer and the radiation power is controlled by the indicator. The applicator has metal case that was divided into two hermetic cavities. The cavity from the slit side was filled with the water and the bottom border of this cavity was covered by thin rubber diaphragm which is virtually an applicator aperture. Water bolus of about 9 mm in thickness assured matching of the radiator with the object. For personnel protection the outer surface of the applicator was covered by an absorbent [6].

The preparation doses for the animals of both age groups were identical and determined according to the advices of their application. The radiation sitting lasted 90 seconds. The applicator touched with the scrotum skin and the total capacity, absorbed into the tissues, was about 15 W.

All the animals were in the same conditions (they were kept in the same house) and the feeding regime was maintained according to the technology that was formed on the farm. The microclimate parameters in the animal house have been controlled: temperature, humidity and air movement speed, the concentration of noxious gases (once a month). For a period of the whole experiment the microclimate parameters have met the standard requirements.

Before the beginning of the experiment body measurements and weighing of bulls have been made. Before the beginning of the basic period the subgroups have been formed finally: each group had five bulls (according to the results of preparatory weighing).

There was no significant difference in live weight of bulls in each subgroup, within the age groups, and there wasn't maximum departure from the average value in the group more than 7,5%. There was the same within the subgroups.

## RESULTS

The highest gain weight under the influence of fitostimulators was observed in younger bulls having the lowest live weight. It was 10–15% higher than in bulls of control subgroup, but the above values in bulls of older age slightly exceeded the values of the control subgroup.

Changes in the live weight for younger bulls, testicles of which were subjected to irradiation, correspond to the changes of that in bulls of younger subgroups that received fitostimulators, but the value was a little less in its absolute amount.

Value of gain live weight in bulls of older age with the maximum live weight, testicles of which were subjected to irradiation exceeded the values of the control subgroup at 15–17%.

Conformity analysis of distribution of live weight in bulls with normal law was carried out before the beginning of statistical treatments of results according to age groups.

The consent criterion  $\chi^2$  was used for examination. The checking results are given in table 1.

**Table 1.** The checking results of law of live weight distribution of bulls.

Group	Date					
	2005-11-04	2005-12-24	2006-01-24	2006-02-24	2006-03-24	2006-04-24
6 months	<b>B</b> <i>q = 30%</i>	<b>D</b> <i>q = 1%</i>	<b>C</b> <i>q = 10%</i>	<b>D</b> <i>q = 1%</i>	<b>B</b> <i>q = 30%</i>	<b>B</b> <i>q = 30%</i>
8 months	<b>B</b> <i>q = 30%</i>	<b>A</b> <i>q = 70%</i>	<b>A</b> <i>q = 70%</i>	<b>A</b> <i>q = 80%</i>	<b>B</b> <i>q = 30%</i>	<b>B</b> <i>q = 30%</i>

Notes: A – The law of distribution coincides with the normal one, even as well as possible; B – The law of distribution doesn't contradict the normal one; C – The law of distribution differs from the normal one considerably; D – The law of distribution differs from the normal one very distinctly; q – The level of importance, %.

At the beginning of the experiment the law of distribution corresponds to the normal one for the bulls of both groups. Then it becomes very distinctly from the normal law for the bull of younger age group (6 months) and by the end of the experiment it conforms to the normal law again.

The law of distribution of live weight in bulls of older group (8 months) begins to change by the end of the experiment. The most important changes are in the subgroup which was influenced by microwave hyperemia – there is sudden increasing of live weight in the most developed bulls in this subgroup.

The change character of the law of distribution is shown in picture 1. In this picture the distribution diagrams of live weight of bulls are shown. The selective dispersion values, defined in suitable time moment are given along the abscissa axis and conventional sings of bulls are shown in table 2. The same symbols were used for the designation of bulls which had the same live weight at the beginning of the experiment.

**Table 2.** Conventional signs of bulls

Subgroup	MH-6					H-6					WGC-6					K-6				
Number	3280	3201	3243	3348	3303	3265	3388	3202	6219	3241	3318	3290	3203	3334	3255	3340	3304	3205	3228	3293
Live Weight	157	165	170	175	180	160	166	168	171	175	160	166	168	171	175	165	169	172	175	179
Sign	<b>1</b>	<b>S</b>	<b>M</b>	<b>L</b>	<b>5</b>	<b>1</b>	<b>S</b>	<b>3</b>	<b>M</b>	<b>L</b>	<b>1</b>	<b>S</b>	<b>3</b>	<b>M</b>	<b>L</b>	<b>S</b>	<b>M</b>	<b>3</b>	<b>L</b>	<b>5</b>

Note: Subgroup designation: letters (MH – effect of microwave hyperemia, H – preparation “Humosvit”, WGC – an extract of wheat grass creeping, K – control); figures – age of bulls at the beginning of experiment, months.

The average values of gain live weight of bulls in the control subgroup are given in table 3. To determine the trustworthiness of difference in the average values of gain live weight in each subgroups, taking into account of the distribution law, has been used the criterion by White or (T – criterion). The important differences among the average values of live weight of bulls in each subgroup haven’t been stated for the period of the whole experiment.

2005-11-04									
MH-6	<b>1</b>			<b>S</b>		<b>M</b>		<b>L</b>	<b>5</b>
H-6		<b>1</b>		<b>S</b>	<b>3</b>	<b>M</b>		<b>L</b>	
WGC-6		<b>1</b>		<b>S</b>	<b>3</b>	<b>M</b>		<b>L</b>	
K-6				<b>S</b>	<b>M</b>		<b>3</b>	<b>L</b>	<b>5</b>
			-1,2			0		1,2	s

2006-02-24									
MH-6	<b>1</b>		<b>S</b>	<b>M</b>		<b>L</b>	<b>5</b>		
H-6						<b>M</b>			
						<b>3</b>			
						<b>S</b>			
						<b>1</b>	<b>L</b>		
WGC-6						<b>M</b>			
						<b>3</b>			
				<b>1</b>		<b>S</b>	<b>L</b>		
K-6							<b>5</b>		
				<b>S</b>	<b>M</b>	<b>3</b>		<b>L</b>	
			-1,2			0		1,2	s

**Picture 1.** The diagram of distribution of live weight of bulls

Comparative change quantities of average values of bull gain live weight in the experimental subgroups with respect of animals of control subgroups are given in table 3. The levels of importance for these differences were also given there.

**Table 3.** Comparative changes of average values of gain live weight

Subgroup, designation	Animal number, head	The period of the experiment				
		Value of change, %				
		2005-11-04 2005-12-24	2005-12-24 2006-01-24	2006-01-24 2006-02-24	2006-02-24 2006-03-24	2006-03-24 2006-04-24
MH-6	5	<b>-8</b>	+3	+2	+6	+5
H-6	5	<b>+10</b>	<b>+8</b>	+5	-6	+2
WGC-6	5	<b>+13</b>	+7	-3	-4	-2
K-6 – average values of gain live weight, g.	5	760	760	750	740	720
MH-8	5	-3	+9	<b>+15</b>	<b>+15</b>	<b>+14</b>
H-8	5	<b>+10</b>	+6	+1	+2	+5
WGC-8	5	<b>+15</b>	+7	<b>-5</b>	-3	0
K-8 – average values of gain live weight, g.	5	730	730	740	740	730

Notes: Level of importance  $q = 5\%$  – bold and color; The important differences haven't – italic.

In each group of bulls by the formation of subgroups the animals which live weight was practically the same, have been selected. These animals were distributed among the subgroups evenly and as a result it was found that each group received three heads (in table 2 they are marked off by special symbol).

Incomplete square – law equations of regression for three factors which of them were on two levels have been determined according to the results of investigations (indices values of these animals have been used).

The first factor  $x_1$  – research preparation. The second factor  $x_2$  – age of animal. The third factor  $x_3$  – level of animal development (value of live weight of bull at the beginning of the experiment). The low level of the factor: parameters of bulls with the minimum live weight, and upper one: parameters of bulls with the maximum live weight (from selected three animals which have been distributed among the subgroups).

As a result of statistical treatment (at the level of importance at 5%) for the gain live weight of bulls the following equations have been received:

$$y = 750 - 8x_1 - 17x_2 + 8x_3 + 8x_1x_3$$

for the preparation "Agroperon";

$$y = 766 + 24x_1 - 17x_2 - 8x_3 - 24x_1x_3$$

for the preparation "Humosvit";

$$y = 774 + 32x_1 + 16x_1x_2 + 16x_3$$

for the tissue preparations formed as a result of microwave hyperemia effect.

The value control of the gain live weight of bulls defined by these formulae and determined during this experiment for the animals with average value of live weight has been shown that the divergence wasn't more than 2,5%.

Cellular and humoral parameter of resistance, an albuminous spectrum blood serum corresponded to the character of live weight change.



## CONCLUSION

From our point of view, the results of this experiment confirm beneficial influence of BAS on the efficiency and resistance of bulls very well. The results of table 3 and the equations of regression are evidence of these. But the presence of important effect of interaction in the equations shows, that the using effectiveness of researched BAS will greatly depend on the age of animals and the level of their development.

The changes of the law of distribution of bull live weight in the younger group take place as a result of influence of BAS – the gain live weight of bulls which were backward in their development in the past, but which were give “Agroperon” and “Humosvit” increases very rapidly. The important changes were not registered in the subgroup MH because at age of 6 months the formation of sexual system in bulls was not completed.

As soon as the influence of preparations has finished, the law of distribution became normal again – the bulls returned to the conditions where the gain live weight became to depend on the great amount of factors again, each of them has an insignificant influence. From our point of view, it confirms by the character of changes of bull gain live weight of the subgroups WGC (taking “Agroperon” – the preparation influencing on the protein metabolism). The changes in this subgroup began earlier, proceeded more rapidly and stopped earlier.

From our point of view, the changes in bulls of the older group can be explained by the influence of tissue preparations formed by the influence of microwave hyperemia. The maximum effect was observed in the most developed bulls which sexual system was completely formed. Vegetable preparations have had a positive influence which was observed in the “leveling” of the law of distribution of live weight – the gain live weight in bulls of experimental group was more than in bulls of control group though the important distinctions haven’t been determined.

The fact is that the difference of live weight values of bulls of the same age, which was by the group formation, explains the difference of the development levels, is a consequence of Chirvinski-Maligonov’s law. The growth has a staged uneven character and at the same time it has the conformity to natural law of continuous growth as an interactive process.

The normalization of metabolism processes is one of the displays of influence of BAS on the objects, especially by the long term use. The overwhelming majority of animals were enveloped by this process in this case [3]. The influence was non-permanent in this experiment and the effect was observed for a period of several months. In case of microwave hyperemia application the positive effect began to affect by the end of this experiment. At first even the lowering of gain live weight in the subgroups MH was observed.

We think that the substances presented in the preparation composition or formed after microwave hyperemia “started up” the mechanism regulating the animal growth or influenced on the organs controlling by this process. It’s coordinated with conceptions of modern genetics about the mechanism of “gene engaging” very well.

It’s necessary to mention that by the using of BAS for increasing of animal efficiency, it’s necessary to form the groups of definite development levels preliminarily and to correlate with the preparation using according to age of animals and their development level.

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## BEHAVIOUR OF DAIRY COWS IN THE WAITING AREA OF LARGE UNINSULATED COWSHEDS

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### SUMMARY

The objective of the current study was to investigate dairy cows' behaviour in the waiting area (WA) of the milking parlour (MP). Some cows have to wait for milking up to five hours per day, which may considerably influence their welfare.

The experiments were carried out at three large uninsulated loose housing cowsheds. In the WA less than 2% of cows were engaged in grooming, vocalization, mounting, licking and conflict; up to 8% of cows showed exploring behaviour. The percentage of ruminating, grooming and exploring cows in the WA increased, whereas the percentage of vocalizing, licking and conflict activities decreased. The cows of the last milking groups can be considered the lowest rank in the feeding group; their possibilities to behave normally are most limited.

**Keywords:** dairy cow, behaviour, waiting area, loose housing, large cowshed, milking order

### OBJECTIVE

Nowadays uninsulated loose housing cowsheds with automatic systems are gaining popularity in the whole world because of low building cost and the environment that safeguards the animal welfare. The loose housing of dairy cattle has developed rapidly in Estonia since the 1990s (Veermäe et al., 2001). The first large uninsulated cowshed was introduced in 2002, now about 30% of the cattle are kept in uninsulated cowsheds with more than 300 dairy cows. However, some concern has been expressed over the systems in which the cows are housed throughout the year, as the behavioural restriction implicit in these systems is associated with poor welfare (Haskell et al., 2003). The current study focused on the cows' possibilities to behave naturally in the WA.

### MATERIAL AND METHODS

The experiments were carried out at three large uninsulated loose housing cowsheds. Cowshed I was introduced in 2003. In 2004 there were 519 dairy cows with milk production of 6374 kg. Cows (Estonian Holstein) were in 4 feeding groups and milked in 2\*12 DeLaval tandem milking parlour two times per day. The size of the waiting area was 171.5 m<sup>2</sup>.

Cowshed II was introduced in 2002. In 2004 there were 561 dairy cows with milk production of 7916 kg. Cows (Estonian Holstein and Estonian Red) were in two feeding groups and milked

in 2\*20 Strankgo tandem milking parlour three times per day. The size of the waiting area was 196 m<sup>2</sup>.

Cowshed III was introduced in 2004. There were 357 dairy cows (Estonian Holstein and Estonian Red) in 6 feeding groups with milk production of 7675 kg. Cows were milked in 2\*12 DeLaval tandem milking parlour three times per day. The size of the waiting area was 140 m<sup>2</sup>.

Cow groups were defined as follows (figure 1)

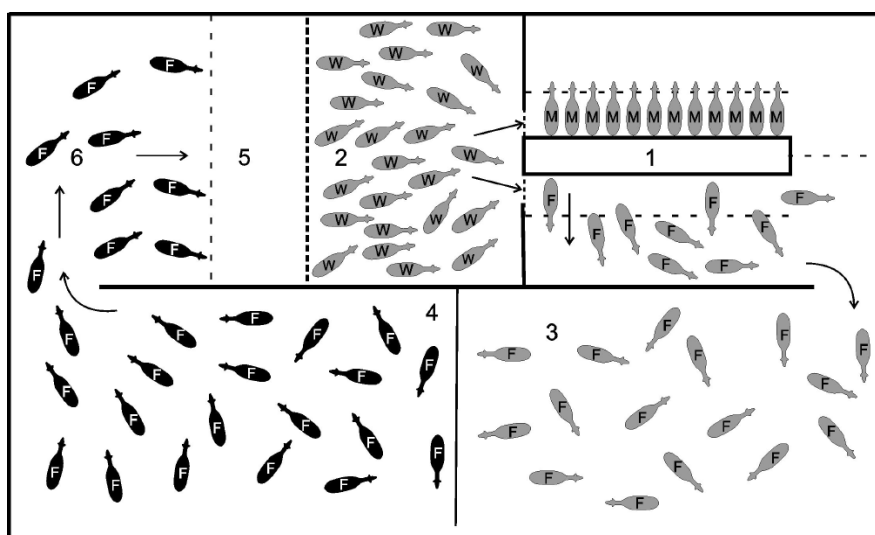
Feeding group – a group of cows in the WA before the start of milking. This group has fixed size and is kept separately according to the feeding-management system in the cowshed.

Waiting group – a group of cows in the WA during milking. The size of the waiting group decreases in the course of milking while subsequent milking group passes the MP.

Milking group – a group of cows on either left or right side of the MP during one milking round.

Milking round – milking of left and right milking groups on the MP (order number).

In every cowshed observations were carried out on the waiting area during three milking. In total 266 milking groups and 3522 dairy cows were observed (cowshed I 121 milking groups and 1388 cows; cowshed II 54 milking groups and 1062 cows, cowshed III 91 milking groups and 1072 cows).



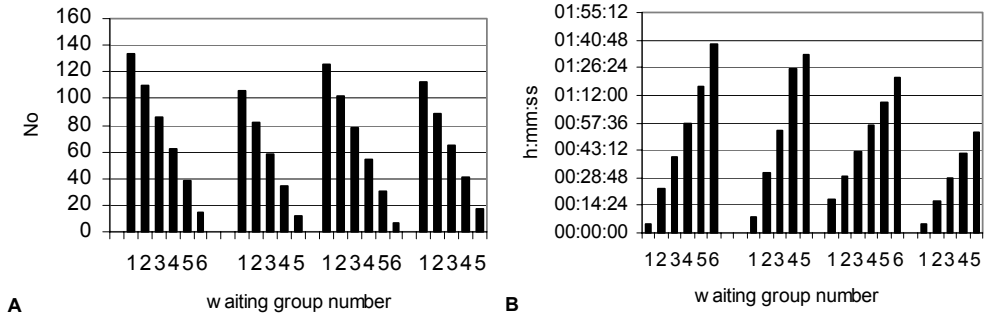
**Figure 1.** Feeding, milking and waiting group cows

Cows' activities (falling down, grooming themselves, licking other cows, mounting, vocalizing, exploration, conflict), stockperson's activities – talking to animals (vocal) and touching and the usage of cows' mover (push) were registered continuously. The number of ruminating cows was registered every 10 minutes.

Behavioural data were calculated per “**waiting group**” – “the number of cows” in the waiting area per time interval between milking rounds (average for left and right side of MP). Cows' activities were characterized by the number of occurrences per group (%) and per animal in the WA; rumination was characterized by % of animals. Statistical analysis was carried out using the EXCEL.

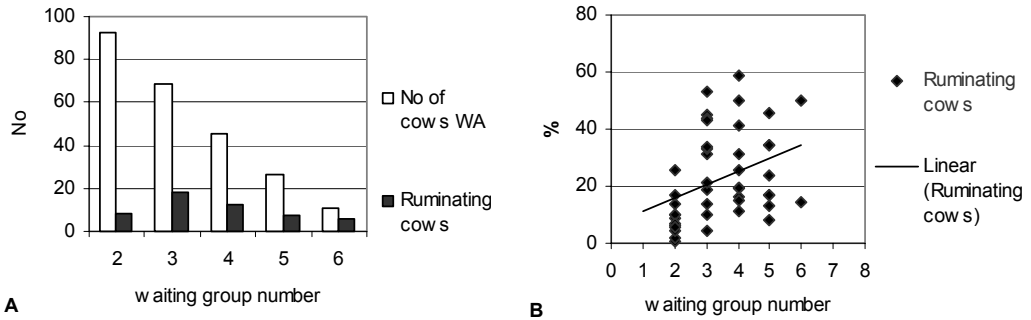
**RESULTS AND DISCUSSION**

No of cows in the WA in time decreases according to the MP size, whereas the waiting time increases (figure 2).



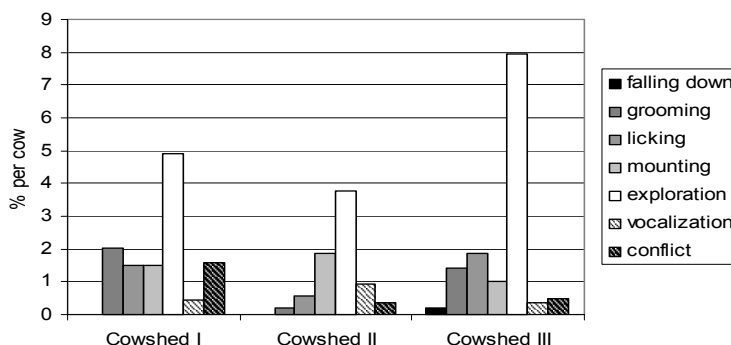
**Figure 2.** A. The number of cows in the WA (cowshed I); B. Cows' waiting time in the WA (cowshed I)

The percentage of ruminating cows in the waiting group in the WA increased in all cowsheds as milking proceeded. However, about 1/3 of all cows were ruminating in cowsheds I and II; in cowshed III 52.8% of cows were ruminating (figure 3).



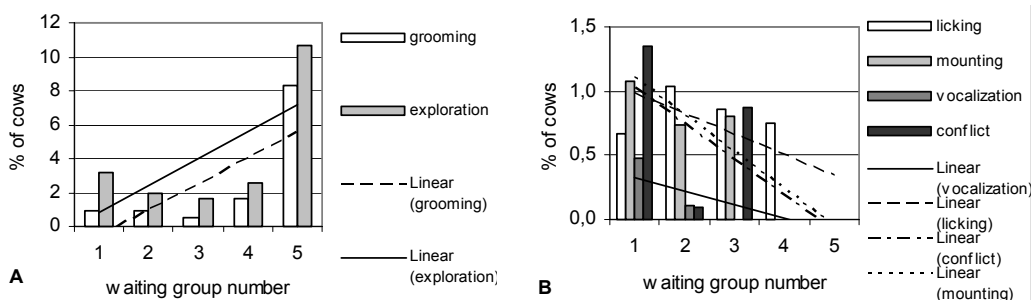
**Figure 3.** A. Total number and number of ruminating cows in the WA (cowshed I); B. Percentage of ruminating cows in the WA (cowshed I)

The activity of cows in the WA was low in all cowsheds. Less than 2% of all the cows were engaged in different activities, except for exploration behaviour (figure 4).



**Figure 4.** Cows' activities in the waiting area per all cows

The number of conflicts was the highest in cowshed I, where 1.4% of the cows of the second waiting group were engaged in conflicts. There were also lickings (subdominant cows' behaviour toward dominant cows) in the first groups (figure 5B). No lickings and conflicts were observed in the last group. Mounting and vocalization activities also had a decreasing tendency. Cows in the last groups performed more exploration and grooming activities (figure 5A). During the observation period only some falling down incidences were observed in cowshed III, where the cows had been only one month.



**Figure 5.** A. Increasing activities in the WA; B. Decreasing activities in the WA

A prominent feature of the social system of dairy cattle is the consistent order of entry into the milking parlour (Rathore, 1982). Cows with low dominance values are forced to spend more time waiting for milking. At the same time, cows' motivation to be milked is not very high. Individual cows may find milking either positively or negatively reinforcing, but overall, the motivation to be milked is weak. Food is significantly more rewarding than milking (Prescott et al, 1998). Geri and Hama (2003) compared the behaviour of Holstein-Friesian cows when entering the milking parlour and at the feeding trough. There was no correlation between the entrance order to the milking parlour and the dominance order at the feeding trough. The younger cows were dominant when entering the milking parlour, and the older, heavier cows at the feeding trough.

The time that the cows spend lying as opposed to standing is of interest both from the cow's and the dairy farmer's point of view (Österman and Redbo, 2001). High producing dairy cows spend about 40–50% of the day lying down and adequate rest is necessary to ensure high

production. Preferred lying time for cattle is 10 hours per day. Belonging to the first milking rounds gave certain priorities to the cows: less standing time in the WA and after returning to the cowshed unlimited access to the feeding table and stalls.

In contemporary large loose housing cowsheds WA is the area, where the cows' possibilities for normal behaviour are the most limited: locomotion activity is strongly restricted and behaviour is controlled by dominance order. Cows in the WA have no possibilities to lie down or eat. Limited space per cows inhibits locomotion and social activity. Dairy cows are close together, there are no possibilities for low ranking cows to withdraw from aggression or to leave the area.

The cows' possibilities for normal behaviour in WA were limited in all cowsheds (figure 4). The most prevalent activity was rumination (figure 2). Only healthy and unstressed cattle will ruminate normally (Lidfors, 1996). In current study up to 52% of cows were ruminating in the WA of cowshed III, where the feeding group size was the smallest, waiting time the shortest and space for one cow the biggest. In cowsheds I and II only 1/3 of cows found the WA comfortable enough to perform ruminating activity there. Low percentage of ruminating cows in the WA indicated uncomfortable environment.

It is impossible to distinguish between the cows from different milking groups in the WA. Therefore the results of observations represent "substructured" image: what changes in cows' group behaviour can be found after the each milking group had left the WA, and which activities are representative for the last waiting groups. Despite of some increase in space per cow (2...3 → 4...5 m<sup>2</sup>), the cows' activity remained low. The same tendencies in activity pattern were found in all cowsheds. It appeared that the cows' social activity decreased: the number of conflicts and lickings dropped, there were no lickings and conflicts in the last waiting groups. The same trend was followed in mounting and vocalization activities (except mounting activity in cowshed II). At the same time exploring and grooming activities increased. The last WA cows that regularly had to wait for milking for several hours per day were calm and patient in their behaviour, exploring and self-grooming activities were dominant. They were not more reluctant to milking.

From the point of welfare waiting area is a critical part of technology at large loose housing cowsheds. It is functioning as a "sorting unit", where "problem" cows are sorted into last milking groups. Cows, which are more or less voluntarily in the last milking groups, can be considered as the lowest rank in the group; their normal behaviour possibilities are the most limited and therefore it is reasonable to focus welfare evaluation on this group on animals.

## CONCLUSIONS

In the waiting area less than 2% of cows were engaged in grooming, vocalization, mounting, licking and conflict; up to 8% of cows were engaged with exploring behaviour. The percentage of cows' ruminating, grooming and exploring activities in the WA increased in connection with the decrease of waiting group size whereas the percentage of vocalization, mounting, licking and conflict activities decreased.

Cows, which are more or less voluntarily in the last milking groups, have fewer possibilities to behave normally, therefore it is reasonable to focus on these cows in welfare evaluations.

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## ACKNOWLEDGEMENTS

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## THE BACTERIAL FLORA IN THE TEAT DUCT OF EWES CAN PROTECT AGAINST AND CAN CAUSE MASTITIS

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### ABSTRACT

**Objective.** We carried out two experiments to study effects of bacterial flora into ovine teat duct to pathogenesis of mastitis. 1st experiment. 32 ewes were allocated into group A (ewes with coagulase-negative staphylococci [+++ growth] in the teat duct), B (ewes with coagulase-negative staphylococci [+ growth] in the teat duct) or C (ewes with *Bacillus* spp. in the teat duct) and subdivided into A1, B1, C1 (n=4; challenged by deposition of 1.250 cfu of *Mannheimia haemolytica* into the teat duct) or A2, B2, C2 (n=4; used as uninoculated controls); group D (n=8) had ewes with no bacteria in the teat duct, challenged also with *M. haemolytica*. There were less bacteriological isolations of flora ( $p=0.018$ ) and challenge ( $p<0.05$ ) organisms from A1 than from A2 and D ewes; pathological findings in A1 (summed up lesion score: 27/64) ewes were less severe than in D (summed up score: 36/64) ewes ( $p=0.038$ ). No such findings were evident with B or C ewes ( $p>0.4$ ). 2nd experiment. Ewes (groups E and F, n=6) with coagulase-negative staphylococci (+ growth) in the teat duct, were used; ewes (group G, n=6) with no bacteria in the teat duct, were included. Teat chapping was applied in E and G ewes. All E ewes developed acute clinical mastitis within 24 h after chapping, although no challenge had been carried out; there were more bacteriological isolations of flora from E than from F or G ewes ( $p<0.001$ ); pathological findings in E (summed up lesion score: 28/32) were more severe than in F (summed up lesion score: 3/32) or G (summed up lesion score: 14/32) ewes. **Conclusions.** We conclude that staphylococcal flora present in high numbers within the teat duct of ewes, provides some protection against invading bacteria. In case of decreased defence mechanisms in the teat, that flora can invade the mammary parenchyma and cause clinical mastitis. **Acknowledgments.** The project is co-funded by the European Social Fund & National Resources – EPEAEK II-PYTHAGORAS.

### INTRODUCTION

The significance of the ovine teat as a defence mechanism against intramammary infections has been established [20]. Clinically healthy teats provided a substantial protection against *Mannheimia haemolytica* intramammary infection. Deposition of either of two isolates of *M. haemolytica* into the teat duct did not result to clinical mastitis, although an inflammatory reaction had been elicited. During that study, we observed lymphoid nodules at the border between teat duct – teat cistern; we postulated that these structures might play a protective role, as in histological sections from teats inoculated with the bacteria; they were hyperplastic with germinal activity. Apart from that, the bacterial flora residing into the teat duct of healthy ewes may also contribute to the protective role of the teat. In field studies, isolation of bacteria from clinically

healthy sheep teats was associated with observations of lymphoid nodules with germinal activity [23].

Coagulase-negative staphylococci are the principal organisms present as bacterial flora into sheep teats [10]. As staphylococci are confirmed aetiological agents of ovine mastitis [8], one may suggest that perhaps and under certain circumstances, flora organisms can also cause mastitis.

However, the above hypotheses have not been tested experimentally. The objective of the work presented in this paper was to investigate the role of the bacterial flora in the teat duct of ewes. Initially, we explored possible interactions between teat duct bacterial flora and invading microorganisms; subsequently, we studied whether teat lesions may predispose ewes to clinical mastitis caused by teat duct bacterial flora.

## MATERIALS AND METHODS

### Experimental design

#### – Overview

Two experiments were performed during this study. They were carried out under a licence for experimental procedures obtained from the Greek Ministry of Agriculture. In the first experiment, clinically healthy sheep teats with bacterial flora (coagulase-negative staphylococci or *Bacillus* spp.) into the duct were inoculated with an isolate of *M. haemolytica* (strain VSM08L). This strain had been isolated in Greece and was found to cause mastitis in ewes when inoculated directly into the gland cistern, whilst deposition into the duct or the cistern of clinically healthy teats resulted to subclinical mastitis [20]. The identity of the organism was initially established by means of conventional bacteriological techniques [6]. In the second experiment, clinically healthy sheep teats with bacterial flora (coagulase-negative staphylococci) into the duct were subjected to skin chapping lesions; no challenge was performed.

#### – Deposition of *M. haemolytica* into bacteriologically positive sheep teat ducts: Experiment I

Twenty-four, 3- to 5-years-old, Karagouniko-breed lactating ewes were included in the experiment. For selection of the ewes three, 2-day interval, examinations and samplings were carried out. Initially, a thorough clinical examination was carried out; special attention was paid to their mammary glands and teats, which were examined as described before [9, 20]. A sterile plastic fine catheter 2 mm long was inserted into the teat and moved from left to right, in order to sample the mucosa [21]. Then, mammary secretion samples (10 to 15 mL) were obtained. Selection of animals was based on concurrent presence of the following criteria at all three samplings: (i) clinically healthy mammary glands and teats; (ii) no bacterial isolation from mammary secretion; (iii) secretion CMT negative with minimal number of leucocytes in Giemsa-stained secretion films; (iv) bacterial isolation from teat catheter of one teat (left or right) in pure culture; (v) no bacterial isolation from teat catheter of the other teat. Allocation of animals into groups was carried out as follows; group A (n=8): isolation of coagulase-negative *Staphylococcus* sp. in heavy growth (+++), group B (n=8): isolation of coagulase-negative *Staphylococcus* sp. in mild growth (+), group C (n=8): isolation of *Bacillus* spp. in heavy growth (+++). Of the eight ewes allocated into each group, 4 (subgroups A1, B1, C1) were challenged and 4 (subgroups A2, B2, C2) were used as uninfected positive controls. Additionally, a group D (n=8) was also included in the experiment. Lambs of these ewes were weaned 18 days after lambing. No bacteria

were isolated from any teat catheter or mammary secretion samples obtained. These were used as inoculated negative controls. After selection, all animals were hand-milked thrice daily. Ewes were examined again and samples were collected as above, on the day of inoculation (D0), which was carried out as described by Mavrogianni et al. [20]. Ewes in A1, B1, C1 and D were challenged 2 mm deep into the teat by means of a sterile plastic fine catheter (Abbocath; Abbott Laboratories Inc., Abbott Park, IL, USA) 20 G. In the other teat of these ewes, 0.2 mL of sterile PBS was injected 2 mm deep into the teat. In ewes of subgroups A2, B2 and C2, 0.2 mL of sterile PBS was injected 2 mm deep into both teats. Ultimately, the teats of the ewes into each group were naturally infected (NI) and/or challenged (CH) as follows; subgroup A1, B1, C1: one teat NI+/CH+, the other teat NI-/CH-; subgroup A2, B2, C2: one teat NI+/CH-, the other teat NI-/CH-; group D: one teat NI-/CH+, the other teat NI-/CH-.

– Artificial skin chapping on sheep teats with bacteriologically positive duct: Experiment II

Twelve, 3- to 5-years-old, Karagouniko-breed lactating ewes were included in the experiment. For selection, the same procedures and criteria as in Experiment I were applied. Allocation of animals into groups was carried out as follows; group E (n=6): isolation of coagulase-negative staphylococci in mild growth, group F (n=6): isolation of coagulase-negative staphylococci in mild growth. Additionally, a group G (n=6) containing ewes with no bacteria in the teat duct was included in the experiment; their selection was carried out as above and they were used as negative controls. After selection, the animals were hand-milked thrice daily. Then, the lower 3.0 to 3.5 cm of both teats of group E ewes or one teat of group G ewes were immersed into a 1 N solution of NaOH for 1 min; the procedure was repeated on the following day (D-1 and D0). The resulting chapping was scored according to the standards described by Fox et al. [7] and Mavrogianni et al. [22]. Ultimately, the teats of the ewes into each group were naturally infected (NI) and/or chapped (CP) as follows; group E: one teat NI+/CP+, the other teat NI-/CP+; group F: one teat NI+/CP-, the other teat NI-/CP-; group G: one teat NI-/CP+, the other teat NI-/CP-.

### **Post-inoculation/chapping examinations**

After challenge or chapping, detailed clinical examination of the mammary glands and teats was carried out daily. Teat catheter samples and mammary secretion samples were collected. All samples were cultured onto Columbia blood agar; the media were incubated aerobically at 37 °C for up to 72 h. The CMT was carried out in secretion samples, as described before [11]. Secretion films were stained by the Giemsa method. Ewes were euthanized on sequential time-points. Dissection of the mammary glands and the teats started immediately; it was carried out as described before [20]. Scrapings from each of the two sites sampled in each teat, as well as parenchyma samples were examined by conventional bacteriological techniques [6]. Identification of staphylococci was carried by means of API-Staph SYSTEM quick identification strips (BioMerieux, Marcy-l'Etoile, France) Conventional histopathological techniques were employed.

### **Data management and analysis**

A scoring system previously developed and described [22] was used and numerical values were assigned for the pathological findings in the experimental animals. A separate score (0–4 scale)

was given for macroscopic and for histological findings in the teat and the mammary gland; these were then added to a 0–16 scale to produce a pathology score for the findings in each ewe.

Statistical analyses were performed in Minitab 14 (Minitab Inc., State College, PA, USA) and Epi-Info 6 (CDC, Atlanta, GA, USA). For analysis, the proportion of positive bacteriological and CMT results between the different groups / subgroups has been compared by using the Chi-square test or the Fisher Exact Test, as appropriate. Total pathology scores were compared using the Friedman Test using each day's total score as the unit and with group as "Treatment" and day number as "Block". Exact binomial Confidence Intervals (CI) for proportions were calculated. Statistical tests were 2-Sided.

## RESULTS

### Pre-inoculation/pre-chapping examinations

The mammary glands and the teats of all ewes were clinically healthy before challenge. The teats were soft with no external abnormalities. All selection criteria were fulfilled in the animals used. In Experiment I and Experiment II, bacteria recovered from teat duct catheter samples met the allocation criteria. In Experiment III, no bacteria were isolated from the mammary secretion or the teat duct catheter samples obtained.

### Post-inoculation/post-chapping clinical, bacteriological and cytological findings

– Deposition of *M. haemolytica* into bacteriologically positive sheep teat ducts: Experiment I  
None of the ewes in subgroup A1, B1 or C1 developed clinical mastitis. From the NI+/CH+ side, *M. haemolytica* was isolated: in total, from 16/32, 24/32, 25/32 samples from A1, B1, C1 ewes, respectively; additionally, the initial bacterial flora was also isolated from duct, but not from secretion, samples: in total, from 10/32, 14/32, 15/32 samples. The CMT increased (>"1"). None of the ewes in subgroup A2, B2 or C2 developed clinical or subclinical mastitis. From the NI+/CH- side, only the initial bacterial flora was isolated from duct, but not from secretion, samples: in total, from 16/32 samples from ewes of each subgroup. The CMT remained negative (<"1"). None of the ewes in group D developed clinical mastitis. From the NI-/CH+ side, *M. haemolytica* was isolated: in total, from 49/64 samples. The CMT increased (>"1"). No clinical signs were observed in any of the NI-/CH- sides (A, B, C, D ewes). No bacteria were recovered these. The CMT was negative. Details in Table 1.

– Artificial skin chapping on sheep teats with bacteriologically positive duct: Experiment II  
All ewes in group E developed systemic and mammary signs. The teats became chapped to score "2" to "3". *Staphylococcus* spp., same species as originally (before chapping) recovered from the teat duct catheter sample, were isolated in pure culture: in total, from 71/72 samples. The CMT increased ( $\geq$  "2"). Control teats (NI-/CP+) of ewes of group E remained chapped to a score "2" to "3"; no clinical findings characteristic of mastitis were observed. No bacteria were recovered. The CMT was mildly positive (score "1"). None of the ewes in group F developed clinical or subclinical mastitis. From the NI+/CP- side, only the initial bacterial flora was isolated from duct, but not from secretion, samples: in total, from 36/72 samples. The CMT remained negative (<"1"). The chapped teats of ewes of group G were scored "2" to "3". No mastitis was observed. From the NI-/CP+ side, no bacteria were recovered from any duct or secretion samples: from 0/72 samples. The CMT was positive (score "1"). No clinical signs were observed in any of the

NI-/CP- sides (F, G ewes). No bacteria were recovered. The CMT was negative. Details are in Table 1.

**Table 1.** Cumulative bacteriological findings and CMT results in samples after challenge of ewes during the three Experiments.

	Experiment I: subgroups						
	A1	A2	B1	B2	C1	C2	D
Bacterial isolation							
D-F <sup>a</sup>	10/16 <sup>b</sup>	16/16	14/16	16/16	15/16	16/16	0/32
D-Mh <sup>a</sup>	10/16	0/16	14/16	0/16	15/16	0/16	29/32
S-F <sup>a</sup>	0/16	0/16	0/16	0/16	0/16	0/16	0/32
S-Mh <sup>a</sup>	6/16	0/16	10/16	0/16	10/16	0/16	20/32
CMT results							
Positive	14/16	0/16	14/16	0/16	14/16	0/16	28/32
	Experiment II: groups						
	E		F		G		
Bacterial isolation							
D-F <sup>a</sup>	36/36		36/36		0/36		
S-F <sup>a</sup>	35/36		0/36		0/36		
CMT results							
Positive	36/36		0/36		32/36		

<sup>a</sup> D-F = teat duct – flora, D-Mh = teat duct – *M. haemolytica*, S-F = secretion – flora, S-Mh = secretion – *M. haemolytica*, D-Ss = teat duct – *S. simulans*, S-Ss = secretion – *S. Simulans*

<sup>b</sup> n/m = positive results out of total animals sampled

### Pathological Findings

– Deposition of *M. haemolytica* into bacteriologically positive sheep teat ducts: Experiment I  
*Post-mortem* bacterial isolations were as follows. From the NI+/CH+ side of A1, B1 and C1 ewes, *M. haemolytica* was isolated: from 4/12, 10/12, 9/12 sites sampled, respectively ( $p=0.024$ ); the initial bacterial flora was also isolated: from 3/12, 8/12, 8/12 sites sampled, respectively ( $p=0.062$ ). From the NI+/CH- side of A2, B2 and C2 ewes, only the initial bacterial flora was isolated: from 7/12, 8/12, 7/12 sites sampled, respectively ( $p=0.89$ ). From the NI-/CH+ side of D ewes, *M. haemolytica* was isolated in pure culture: from 15/24 sites sampled (0.625, 95% C.I.: 0.41–0.81). Statistical comparisons revealed that for A1 vs D,  $p=0.044$ , whilst for B1 or C1 vs D,  $p>0.4$ . No bacteria were isolated from the contralateral side (NI-/CH-) of these ewes. The total pathology scores summed over all days were 27, 33 and 35 for NI+/CH+ side of A1, B1 and C1 ewes ( $p=0.041$ ), respectively (maximum possible: 64). The total pathology scores summed over all days were 8, 8 and 6 ( $p=0.37$ ) for NI+/CH- side of A2, B2 and C2 ewes, respectively (maximum possible: 64). The median total pathology score summed over all days was 36 for NI-/CH+ side of D ewes (maximum possible: 64). Statistical comparisons revealed that for A1 vs D,  $p=0.038$ , whilst for B1 or C1 vs D,  $p>0.6$ .

– Artificial skin chapping on sheep teats with bacteriologically positive duct: Experiment II  
*Post-mortem* bacterial isolations were as follows. From the NI+/CP+ side of E ewes, *Staphylococcus* sp. same species as originally recovered from the teat duct catheter sample, were consistently isolated in pure culture from the teat duct, teat cistern and mammary parenchyma: from 18/18 sites sampled; no bacteria were isolated from the contralateral side (NI-/CP-). From the NI+/CP- side of F ewes, *Staphylococcus* sp. same species as originally recovered from the teat duct catheter, were consistently isolated in pure culture from the teat duct: from 6/18 sites sampled; no bacteria were isolated from the other side (NI-/CP-). No bacteria were recovered either from the NI-/CP+ side of group G ewes: from 0/18 sites sampled or from their other side (NI-/CP-). The median total pathology score summed over all days was 28 for NI+/CP+ side of group E ewes (maximum possible: 32). The median total pathology score summed over all days was 3 for NI+/CP- side of group F ewes (maximum possible: 32). The median total pathology score summed over all days was 14 for NI-/CP+ side of group G ewes (maximum possible: 32). The median total pathology score summed over all days was 14 for NI-/CP+ side of group E ewes (maximum possible: 32). The median total pathology score summed over all days was 0 for NI-/CP- side of ewes of groups G and G (maximum possible: 32).

## DISCUSSION

Previous experimental studies on ovine mastitis have established the protective role of the teat against intramammary infections; Mavrogianni et al. [20] studied the effects of the inoculation of *M. haemolytica* in different sites of healthy, bacteriologically negative teats. The results of that study showed that the ovine teat acted as a barrier against bacteria. During that study, we also suggested that the bacterial flora present in the teat duct of healthy ewes [10] might act competitively against invading bacteria and thus provide one of the defence mechanisms active in the teat.

In the present work, we inoculated bacteriologically positive teats with a *M. haemolytica* isolate, in order to study possible interactions between the bacterial flora and a confirmed mastitis causal agent [1, 2]. Ewes in subgroup A1 and D developed subclinical mastitis. However, recoveries of the challenge organism from the former animals were significantly fewer than from controls, thus suggesting an effect on the challenge strain; furthermore, the severity of the mammary lesions was significantly smaller in A1 than in D ewes. Adherence of *M. haemolytica* on mammary epithelial cells is required for its multiplication and leucotoxin production [32]; based on the present findings, one may postulate that the bacterial flora inhibited that process. No such bacteriological and pathological differences were seen in B1 and C1 ewes; this suggests that the protective effect of bacterial flora was exercised preferentially and only by staphylococcal species present in large numbers within the teat duct.

Bacterial competition is the situation where two bacterial populations compete for multiplication and survival, usually resulting in cell population reduction or impeded growth rate than if the two populations were separated [14]. This was evident in subgroup A1, where a distinct protective effect of the flora was recorded. Both the flora populations and the challenge, invading organism were subsequently recovered from a reduced number of samples than from respective controls. This type of relationship between bacteria occurs when two species compete to occupy a particular site [18, 31]. Rainard and Poutrel [27] have also reported that new infections were less frequent in glands already harbouring a pathogen. All these findings further support the above hypothesis.

Production of antagonistic substances by bacterial flora and competition for necessary nutritional substances between flora and invading organisms [30] are also contributing mechanisms. The direct toxic effects of certain bacterial species against other ones invading the host have also been considered, as flora populations can secure their domination over invading pathogens by producing antibacterial substances [4, 15]. Staphylococcal strains isolated from cows' teat orifice or mammary secretion, have been found to produce bacteriocins and reduce in vitro growth of other pathogens [5, 25].

However, when the microbial equilibrium is disrupted for any reason, it is possible that pathogenicity of the flora strains would increase, leading to disease. Mayrand and Grenier [24] studied bacterial interactions and found that once the intra-bacterial balance was broken, pathological changes were initiated. Under those circumstances, the flora would contribute to development of disease either by facilitating an invader to fully expressing its pathogenicity or even by participating in the infectious process itself in order to establish the pathological findings. The findings of Experiment II clearly indicate that under certain circumstances, the resident bacterial flora can become pathogenic. Ewes in group E rapidly developed acute clinical mastitis without bacterial challenge; the disease was caused by the bacterial flora organisms, which multiplied and ascended to the mammary parenchyma. Lesions observed during this study were typical of staphylococcal mastitis [8]. In this case, the "trigger factor" that led to the equilibrium shift was the teat chapping.

In a recent paper, Mavrogianni et al. [22] provided evidence that teat chapping predisposed ewes to mastitis in cases of new bacterial infections. Chapped teats are considered an increased risk for mastitis [26, 28]. During cold weather, increased incidence of chapped teats has been reported [7]. In ewes, Leyshon [17] has reported that mastitis was more prevalent in cold weather; this could have been the consequence of chapped teats.

In damaged tissues there is reduced responsiveness and defective chemotaxis of neutrophils [3], which cannot withstand the low pH and high temperature in chapped tissues [12, 16]. Additionally, the reduced hydration of chapped skin alters skin microflora, consequently decreasing resistance to bacterial colonization. We thus believe that in these circumstances, depletion of cellular defences consequently to chapping, resulted in shifting of the balance and allowed bacterial invasion and mastitis. One may also suggest that exposure to trauma may cause degranulation and lysis of mast cells, which are active during acute stages of inflammation [13], consequently reducing the defence abilities of the teat.

Perhaps under field conditions and on a longer-term basis, any factors affecting the immune status of the animals, would affect the equilibrium of flora organisms within the teat, thus resulting to mastitis.

In the past, presence of bacterial flora within a mammary gland has been advocated as a means of preventing mastitis in cows [19]. From that viewpoint, preservation of a protective teat duct flora would be useful for prevention of the disease. Nevertheless, an intramammary infection with a microorganism, even in small doses, might result in increased somatic cell counts, tissue damage and adverse production effects [29]. On the other hand also, teats harbouring bacteria can be a source of infection for the mammary gland. Any impediment of the defence mechanisms (local or systemic) may shift the balance and allow the bacteria to multiply, invade the mammary gland and cause mastitis.

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## EFFECTS OF LAMB SUCKING ON THE BACTERIAL FLORA OF TEAT DUCT AND MAMMARY GLAND OF EWES

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### ABSTRACT

**Objective.** We determined differences in bacterial flora populations of teat duct and mammary gland of ewes before and after sucking by lambs; we also evaluated factors potentially affecting these differences. **Methods.** We collected samples of teat duct material (by means of fine [20 G], plastic, sterile 2 mm-long catheters) and mammary secretion from 11 ewes immediately before (< 40 s) and immediately after (<30 s) sucking by their lambs, as well as 120 min later. We processed samples by conventional bacteriological techniques. We compared changes in infection by means of Sign Test. **Results.** We isolated bacteria from 3.5% duct and 1.5% secretion samples before sucking; respective figures after it were 10.6% and 2.0%, and 120 min later 6.8% and 1.5%. We saw differences among duct material samples collected before and after sucking, in 40 cases: from 6 bacteria were isolated only before, from 34 only after ( $p<0.001$ ); respective results for secretion were 4 cases. We saw differences among duct material samples collected after suckling and 120 min later, in 12 cases: 8 and 4 ( $p=0.375$ ), respectively; no changes were for secretion samples. We found neither difference among ewes with single or twin lambs, nor among stages of lactation. Mostly, we isolated staphylococci: 70% of isolates before suckling, 80% of isolates after it, 91% of isolates 120 min later; we also isolated two *Mannheimia haemolytica* strains immediately after sucking. **Conclusion.** Sucking predisposes to entrance of bacteria into the teat duct; however, increased teat duct infections did not result to respective mammary infections. Subsequently, teats overcame the infections. *M. haemolytica* isolated directly after suckling indicates lambs as source of infection.

### INTRODUCTION

It has been repeatedly in studies of ovine mastitis, that sucking by lambs is associated with transfer of microorganisms into the teat duct [12, 19, 22]. There are three different sources of bacteria, which might enter into the teat duct, namely the lamb (mouth, nasopharynx), the ewe (udder skin) or the environment (bedding). These organisms may subsequently ascent to the mammary gland and cause mastitis. The objectives of this investigation were i) to determine differences in bacterial flora populations of teat duct and mammary gland of ewes before and after sucking and ii) to evaluate factors that potentially affect them.

## MATERIALS AND METHODS

### Sampling schedules

Eleven multiparous Karagouniko-breed lactating ewes were included in this study within 5 days after lambing and housed separately throughout. Ewes were selected among animals with no history of mastitis; the California Mastitis Test (CMT) was performed in mammary secretion samples from both glands of these ewes, with five degrees of reaction scores (“negative”, “trace”, “1”, “2”, “3”) as detailed by Fthenakis [9]. Teat duct material and mammary secretion samples were obtained and processed as detailed below. From the ewes selected, seven suckled a single lamb and four suckled twins. Standard husbandry conditions applied throughout the study. The work was carried out under a license for experimental procedures issued by the Greek Ministry of Agriculture, based on EU guidelines. Paired-samples were obtained from both mammary glands of each ewe. Samples were obtained on three occasions weekly, for six weeks (2nd to 7th of lactation). Lambs were separated from their dams for 60 ( $\pm 3$ ) min; during that time no feed, milk or water were provided. Samples (“A”) were collected and within 30 s lambs joined their dams; they immediately ( $< 7$  s) started sucking, whilst ewes were restrained in the standing position. Then new samples (“B”) were collected, by following a different schedule on each of the sampling occasions. For collection of samples “B<sub>1</sub>” (1st sampling occasion of each week), natural termination (i.e., by the lamb or the ewe, without human interference) of a sucking bout was allowed. For collection of samples “B<sub>2</sub>” (2nd sampling occasion of each week), lambs were removed from their dam 3 min ( $\pm 2$  s) after joining her. For collection of samples “B<sub>3</sub>” (3rd sampling occasion of each week), lambs were removed from their dam 1 min ( $\pm 2$  s) after joining her. In all cases, samples were obtained within 30 s. All lambs were observed, to confirm that both teats of the dam had been sucked (including ewes with a single lamb). Finally, further samples (“C”) were collected 120 ( $\pm 5$ ) min after collection of “B<sub>1</sub>” samples.

### Collection and processing of samples

Before sampling, a thorough clinical examination was carried out on the ewes, with special attention paid to their mammary glands and teats [8, 19]. A thorough disinfection with povidone iodine scrub solution was carried out in the teat apex and the lower (1 cm) part of the teat skin. A sterile, plastic, 20 G catheter (Abbocath<sup>®</sup>; Abbott Laboratories Inc., Abbott Park, USA) was used for sampling material from the teat duct. The catheter stylet was taken out and the plastic catheter was cut with a sterile blade to a length of 2 mm. In order to ensure accurate and consistent cutting of the catheter at the desired length, a sterilized ruler was always placed beside the catheter. The whole procedure was carried out under aseptic conditions. The catheter was held by the investigator from the cannula hub; it was inserted into the teat, rolled around the internal teat wall, in order to sample the mucosa, and then withdrawn. Description and validation details of the method were presented by Mavrogianni and others [16]. Subsequently, secretion samples were obtained. The first two squirts were drawn onto the palm of the gloved hand of the investigator and examined for the presence of abnormalities; then, 10 to 15 ml were carefully collected into a sterile container. These procedures were carried out in all samplings. Standard procedures for sampling and processing of samples previously described in detail [8, 19] have been used during the study. Duct material collected on the tip of the catheter and mammary secretion samples were plated onto Columbia 5% blood agar; the media were incubated aerobically at 37 °C for up to 72 h. Throughout this study, all bacteria isolated were identified by using conventional techniques [2,

5]; for staphylococcal identification, the “API-Staph SYSTEM” quick identification strips were also used (BioMerieux S.A., Marcy-l’Etoile, France).

### **Data management and statistical analysis**

The model described in a previous experimental work using paired-samples [18] was employed. Analysis of results was carried out by comparing changes in infection status between “A” and “B” samples and between “B<sub>1</sub>” and “C” samples. Duct material and secretion samples were assessed separately. Statistical significance was assessed by the Sign Test, which allowed for the readings to be paired. Furthermore, comparisons were performed between ewes suckling single or twin lambs, as well as between “B<sub>1</sub>”, B<sub>2</sub>” and “B<sub>3</sub>” samples. Finally, changes between stages of lactation (Stage I: 2nd and 3rd week of lactation, Stage II: 4th and 5th week, Stage III: 6th and 7th week) were evaluated. Analysis of Variance for proportions over time was employed. Data were modelled in Minitab 14 (Minitab Inc., State College, PA, USA). The critical probability was set at  $p = 0.05$ , on a 2-sided null hypothesis of no difference.

## **RESULTS**

### **Clinical findings**

None of the ewes included in the study had a history of mastitis. No bacteria were isolated from any duct material or mammary secretion samples obtained from the ewes before inclusion into the study. All CMT results were negative. Neither changes in mammary secretion, nor mammary abnormalities were detected in ewes sampled during the study. In all cases, lambs started sucking immediately (<7 s) after joining their dam. Both teats of the ewes were sucked. In total, 924 duct material and 924 secretion samples were collected during the study. These were as follows: 396 “A” samples (252 from ewes with a single, 144 from ewes with twins) and 132 each of “B<sub>1</sub>”, “B<sub>2</sub>”, “B<sub>3</sub>” or “C” samples (in each of these, 84 from ewes with a single, 48 from ewes with twins).

### **Effects of suckling on bacterial isolations**

Among “A” samples, 14/396 (3.5%) duct material and 6/396 (1.5%) secretion were bacteriologically positive. Among “B” samples, 42/396 (10.6%) duct material and 8/396 (2.0%) secretion were bacteriologically positive. Among “C” samples, 9/132 (6.8%) duct material and 2/132 (1.5%) secretion were bacteriologically positive (Table 1).

After suckling, there was a significant increase by 200% (from 14 to 42) in infected teat ducts ( $p < 0.001$ ). No effect was found on mammary secretion: from 6 infected samples to 8 ( $p = 0.590$ ). In 40 (10.1%) cases, there was a change of bacteriological status of duct material samples in-between suckling; in 6 cases a positive sample became negative, whilst in 34 cases a negative sample became positive. Changes concerned 12/132 “A” to “B<sub>1</sub>”, 15/132 “A” to “B<sub>2</sub>” and 13/132 “A” to “B<sub>3</sub>” pairs of samples ( $p > 0.540$ ). Changes were observed in 22/252 pairs from ewes with singles and 18/144 from ewes with twins ( $p = 0.346$ ). They were observed in 12/132 pairs from Stage I of lactation, in 12/132 from Stage II and in 16/132 from Stage III ( $p > 0.420$ ). In 4 (1.0%) cases, there was a change of bacteriological status of secretion samples in-between suckling; in 1 case a positive sample became negative, whilst in 3 cases a negative sample became positive. Subsequently (120 min after suckling), there was a decrease by 31% (from 13 to 9) of infected

teat ducts ( $p=0.375$ ). No change was recorded in mammary secretion; 2 infected samples on both occasions ( $p=1.000$ ). In 12 (9.1%) cases, there was a change of bacteriological status of duct material samples during the 120 min after suckling; in 8 cases a positive sample became negative, whilst in 4 cases a negative sample became positive. Changes were observed in 7/84 paired-samples from ewes with singles and 5/48 from ewes with twins ( $p=0.700$ ). Changes concerned 3/44 pairs from Stage I of lactation, 5/44 from Stage II and 4/44 from Stage III ( $p>0.460$ ).

**Table 1.** Bacteriological status of samples from ewes before and after suckling of lambs, classified according to number of suckling lambs or to stage of lactation

	Ewes with a single	Ewes with twins	Stage I <sup>a</sup>	Stage II	Stage III	Total
“A” Samples (before suckling)						
Duct samples	5/252 <sup>b</sup>	9/144	3/132	5/132	6/132	14/396
Milk samples	3/252	3/144	3/132	2/132	1/132	6/396
“B” Samples (after suckling)						
Duct samples	21/252	21/144	13/132	13/132	16/132	42/396
Milk samples	4/252	4/252	3/132	3/132	2/132	8/396
“C” Samples (120 min later)						
Duct samples	5/84	4/48	2/44	3/44	4/44	9/132
Milk samples	1/84	1/48	2/44	0/44	0/44	2/132

<sup>a</sup> Stage I: 2nd and 3rd week of lactation, Stage II: 4th and 5th week, Stage III: 6th and 7th week.

<sup>b</sup> n/m = bacteriologically positive results out of total samples.

### Bacterial identity

Bacteria were always isolated in pure culture. The majority of isolates were coagulase-negative staphylococci (*Staphylococcus epidermidis*, *S. simulans*, *S. xylosus*, *S. chromogenes*, *S. sciuri*, *S. caprae*, *S. schleiferi*). These organisms accounted for 52/65 (80%) and 11/16 (69%) of the total isolates obtained from duct material and secretion samples, respectively. Other organisms isolated were: streptococci, *Escherichia coli*, *Bacillus* spp., *Mannheimia haemolytica*, *Arcanobacterium pyogenes*, *Klebsiella* sp. and *S. aureus* (Table 2).

Both *M. haemolytica* strains were isolated from duct material samples obtained after suckling (a “B<sub>2</sub>” and a “B<sub>3</sub>” sample) from two different ewes; both strains were isolated during the 3rd week of lactation. There were no significant differences in the proportion of staphylococci recovered before suckling (70% of total isolates), after suckling (80% of total isolates) or 120 min later (91% of total isolates) ( $p>0.290$ ). Of the 16 bacteriologically positive secretion samples obtained during the study, in 11 (69%) the same organisms as those from the respective duct material sample, were isolated.

**Table 2.** Frequency of isolation of each bacterial species from duct material or mammary secretion samples collected from ewes

	“A” samples		“B <sub>1</sub> ” samples		“B <sub>2</sub> ” samples		“B <sub>3</sub> ” samples		“C” samples		Total samples	
	D <sup>a</sup>	S <sup>a</sup>	D	S	D	S	D	S	D	S	D	S
Coagulaseve staphylococci	11	3	10	1	13	3	10	2	8	2	52	11
Streptococci	1	2	0	1	0	1	0	0	0	0	1	4
<i>E. coli</i>	1	0	0	0	1	0	1	0	1	0	4	0
<i>Bacillus</i> spp.	0	0	1	0	2	0	0	0	0	0	3	0
<i>M. haemolytica</i>	0	0	0	0	1	0	1	0	0	0	2	0
<i>A. pyogenes</i>	0	1	1	0	0	0	0	0	0	0	1	1
<i>Klebsiella</i> sp.	1	0	0	0	0	0	0	0	0	0	1	0
<i>S. aureus</i>	0	0	1	0	0	0	0	0	0	0	1	0
Total	14	6	13	2	17	4	12	2	9	2	65	16

<sup>a</sup> D: duct material samples, S: secretion samples.

## DISCUSSION

### The experimental model

In previous studies of ovine mastitis, it has been confirmed that the teat is the portal of entry of the causal agents, the most important of which are *Staphylococcus* spp. and *M. haemolytica*, together accounting for over 80% of isolates [4]. Staphylococci are considered to originate from milkers' hands or from the skin of the udder [3, 15]). Scott and Jones [22] and Jones and Watkins [12] proposed that *M. haemolytica* originated from the tonsils of the sucking lambs; however, the hypothesis has never been confirmed. In cows, it has been established that improper milking technique predisposes animals to mastitis. As the teat canal dilates, bacteria can invade into the teat. Its orifice may remain open for up to two hours after completion of milking [14, 26], thus facilitating invasion of bacteria into the teat and subsequent ascent to the mammary gland. Similar findings have been presented in dairy ewes after hand-milking [18]. To the best of our knowledge, the possible role of lamb sucking in transferring bacteria into the teat has not been studied. The experimental model that we used, i.e. paired-samples immediately before and immediately after suckling, minimized the time in-between sampling/suckling/sampling. Thus, it ensured that bacterial isolations reflected true dynamics of infection throughout.

### Dynamics of infection

We found significantly increased teat duct infections after suckling, but no strong evidence of increased intramammary infections. Infection of the teat occurred as soon as 1 min after initiation of suckling. However, there were no significant differences among the three procedures evaluated (“B<sub>1</sub>”, “B<sub>2</sub>”, B<sub>3</sub>”). We should also consider the possibility that some bacteria enter into the teat, but are subsequently withdrawn during the same suckling. In a previous experimental study [19], deposition of pathogenic organisms into the duct of clinically healthy ewes did not result to clinical mastitis; thus the protective role of the intrinsic defenses of the teat was confirmed. In the sequel to that work [17], the same procedure was carried out to teats with natural or

experimentally inflicted lesions and severe clinical mastitis developed soon after challenge. We attributed this to increased colonization of damaged teat skin and to physicochemical changes hindering the normal defensive process of the mammary gland. In the current study, the bacteria entering into the teat duct after suckling provided a “natural” means of inoculation of the teat duct, which resisted invasion upwards into the parenchyma. In the teat of cows, various defense mechanisms have been described, e.g. the keratin lining in the teat duct, as well as leucocytes and non-specific antibacterial proteins in the teat cistern [20, 21]. The present findings provide field corroboration of the protective role of the healthy teats of ewes, especially after application of a challenge factor (i.e., suckling). It is noteworthy that although there were further challenges to the teats, as lambs sucked many times during the 120 min interval (“B<sub>1</sub>” to “C” sample), there was still a reduction in infected teat ducts. This suggests that the majority of bacteria would be exterminated within the healthy teats. This defense mechanism aims at reducing bacterial populations within the teat, thus minimizing possible risk of mastitis. In fact, even natural resident flora within the teat duct may cause clinical mastitis, if teat lesions would subsequently be developed [7]. No differences were observed among ewes suckling a single lamb or twins or among periods of lactation. This is not surprising, because when suckling (i.e., in-between samples “A” and “B”) every teat was exposed to challenge: in ewes with twins, each lamb sucked one teat; in ewes with a single lamb, this sucked both teats. Therefore, teats had equal opportunities for infection, and thus, no significant difference were observed. As mentioned above, sampling procedure, and therefore duration of sucking by lambs, did not appear to have an effect. Therefore, challenge opportunities, corresponding to frequency of suckling, appear to be more important for transmission of bacteria and predisposing to mastitis.

### **Transmission of staphylococci and of *M. haemolytica***

Staphylococci, which entered into the teat duct, might have originated from the skin of the teat or from the lips of the lambs themselves. In fact, Laukova and Marounek [13] have isolated coagulase-negative staphylococci from the upper alimentary tract of lambs, whilst Vautor and others [24] reported nasal carriage of staphylococci in sheep. Staphylococci have been traditionally considered an important mastitis pathogen in dairy ewes, likely originating from the hands of milkers. In an extensive field survey carried out in suckling ewes in Great Britain [10, 11], these bacteria were the primary causal agents of subclinical mastitis, as well as being isolated from cases of clinical mastitis. The present findings confirm that these organisms can be transferred to the mammary gland during suckling and explain their involvement in mastitis in meat-producing breeds of sheep, where no hands touch the teat. *M. haemolytica* was recovered from teat duct material only after suckling. The organism had not been isolated from those sites, seconds before initiation of suckling. This is clear evidence that the organism was transmitted from the lambs to the teat ducts. Given that Al-Sultan and Aitken [1] have reported tonsillar carriage of *M. haemolytica* in up to 100% of healthy lambs, the most probable source of the organism would be the upper respiratory tract of the lambs. This finding confirms the initial hypothesis previously presented by Scott and Jones [22] and Jones and Watkins [12]. We postulate that as the lower part of the teat comes into contact with the pharynx of the lamb [23], the organism is attached thereon, subsequently entering into the duct; perhaps the tongue of the lamb may “push” the bacteria upwards into the duct. Isolation of the organism after short (1 min) suckling activity indicates the speed by which the whole process can take place. Vilela and others [25] have documented *M. haemolytica*'s requirement for attachment on the mammary cells, in order to exhibit its pathogenicity. Perhaps the same sucking activity by the lamb can subsequently

remove from the teat bacterial cells not yet attached onto mammary epithelial cells. Although suckling ewes are frequently exposed to challenge by the organism, incidence risk of clinical mastitis associated with it, has been estimated around 5% [10]. Hence, one may suggest that differences in pathogenicity factors among the various strains are responsible for development of mastitis only in some ewes. Additionally of course, inefficiency of teat defense mechanisms would further contribute.

### CONCLUDING REMARKS

The results provide clear evidence that suckling increases the risk of infection of the teat duct of ewes. Nevertheless, teats were able to withstand and minimize the infection within the next two hours. The results also show that *M. haemolytica* may be transmitted during suckling activity. Maintenance of healthy teats, e.g. free from bites or viral lesions, is important for effective defence mechanisms and consequently, for prevention of mastitis.

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## CHEMICAL COMPOSITION AND ENERGETIC VALUE OF CORN AND SUDAN GRASS SILAGE ADDED WITH MOLASSES AND BOVINE FAECES

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### SUMMARY

The objective was to evaluate the effect of the addition of molasses and bovine feces in the chemical composition and energetic value of corn and Sudan grass silage. Six treatments were evaluated, containing different percentages of molasses and bovine feces. There was only an increase in crude protein content ( $P < 0.05$ ) in corn silage as a result of bovine feces addition. In both forages occurred an increase in net energy of maintenance and in net energy of gain. Treatment consisted of 25% of bovine feces addition improved crude protein and both energies in corn silage. On the contrary, for Sudan grass silage 20% of bovine feces and 5% of molasses addition gave the best result for the same variables.

**Keywords:** chemical composition, energetic value, corn grass, Sudan grass, silage, molasses, bovine faeces

### INTRODUCTION

Corn is the main crop in Sinaloa, according to SAGARPA (2007) during the last two cycles, 2005–2006 and 2006–2007 have been sown more than 400, 000 ha, with a total annual production of at least 4.5 000 000 t in irrigated lands, Sudan grass is the second important grass in Sinaloa. In the case of corn, it has a high energetic value but low protein content and Sudan grass has less quality and is less palatable, and as result of their extensive production in this State, there exist a lot of products to offer to cattle. Therefore, one alternative is to silage the whole plant (plant and young ear or spike) (FEDNA, 2004). This State has several dozen of thousands of milk and meat producing cattle which must be fed and at the same time they produce annually thousand of tons of feces that can be either considered either as an environmental problem or as a by-product to produce solid and liquid composts and in many cases during the last decades to feed cattle, of course with some restrictions (Smith and Wheeler 1979; Uicab-Brito and Sandoval, 2003). The feeding value of a forage product is defined as the capacity to improve animal production (meat, milk, eggs, etc) as result of nutrients availability and its intake by animals (Beever *et al.*, 2000). A way to improve the energetic and protein value of silage is to add not only feces but other products such as molasses, being this a sugar cane by-product (Bhattacharya y Fontenot, 1966; Calvert y

King, 1977; Smith and Wheeler, 1979; Cobos *et al.*, 1988). Having all this into account, the objectives of this research were.

## OBJECTIVES

To evaluate the effect of molasses and bovine feces addition to corn and Sudan grass silage on chemical composition and energetic value.

## MATERIALS AND METHODS

This research was carried out at the Nutrition and Animal Bromatology Laboratory at the Faculty of Agronomy, Universidad Autonoma de Sinaloa, Mexico. Six silages (treatments) were evaluated (Table 1) and prepared according to Archila *et al.* (1991). Samples were oven dried at 60°C for 48 h and then analyzed its chemical components such as: Crude Protein (CP), Hemicellulose (HEMI), Cell Content (CC), Acid Detergent Fiber (ADF), and Neutral Detergent Fiber (NDF) (Goering y Van Soest, 1970; AOAC, 1975). The Energetic Composition or Digestible Energy (DE, Mcal kg<sup>-1</sup>), Net Energy of maintenance (NEm), Net Energy of gain (NEg), Net Energy of lactation (NEl), and Total Digestible Nutrients (TDN %) were also estimated (Jurgens, 1988; Undersander *et al.*, 1993). The statistic analysis consisted of analysis of variance and mean comparisons (P<0.05) in a randomize complete block design (SAS version 9.2, 2004).

**Table 1.** Treatments, ingredients and proportion of each one.

Treatments	Ingredients	Proportion (%)
1	Silage corn	100:00
2	Silage corn + bovine feces	85:15
3	Silage corn + bovine feces	75:25
4	Silage Sudan grass	100:00
5	Sudan grass + bovine feces	80:20
6	Silage Sudan grass + bovine feces + molasses	75:20:05

## RESULTS AND DISCUSION

Chemical composition of treatments is shown in Table 2 where can be seen a great deal of variation among treatments; with the exception of Dry Matter (statistic analysis not shown). Treatment of corn silage (1–3) had the lowest results for ADF and NDF; CP was higher in treatments containing Sudan silage (4–6), the smallest value in this variable and in Dry Matter was for treatment with only silage corn (1). Such results are also shown for ADF and HEMI for this treatment. Sudan silage alone had consistently high results for most of variables, but at the same time the lowest for Hemicellulose. NDF was high in the three treatments containing Sudan silage even with the addition of molasses.

**Table 2.** Chemical composition of treatments.

Treatments	DM (%)	CP (%)	NDF (%)	ADF (%)	HEMI (%)	CC (%)
1	91.25	9.60 <sup>c</sup>	55.83 <sup>b</sup>	40.49 <sup>a</sup>	14.00 <sup>a</sup>	49.05 <sup>b</sup>
2	92.42	12.10 <sup>b</sup>	57.34 <sup>b</sup>	46.31 <sup>b</sup>	10.20 <sup>ab</sup>	47.00 <sup>b</sup>
3	93.67	13.00 <sup>b</sup>	50.17 <sup>a</sup>	47.34 <sup>b</sup>	2.65 <sup>c</sup>	53.00 <sup>a</sup>
4	93.19	14.95 <sup>a</sup>	74.68 <sup>d</sup>	59.66 <sup>c</sup>	14.00 <sup>a</sup>	30.40 <sup>d</sup>
5	94.58	15.45 <sup>a</sup>	65.50 <sup>c</sup>	60.74 <sup>c</sup>	4.50 <sup>bc</sup>	38.05 <sup>c</sup>
6	93.73	14.40 <sup>a</sup>	64.65 <sup>c</sup>	49.55 <sup>b</sup>	14.15 <sup>a</sup>	39.40 <sup>c</sup>
CV*	–	2.24	1.41	2.16	16.41	1.91

\* Coefficient of variation

DM (%) =Dry Matter

CP (%) = Crude Protein

NDF (%) = Neutral Detergen Fiber

FDA (%) = Acid Detergen Fiber

HEMI (%) = Hemicellulose

CC (%) = Cell Content

Table 3 presents energetic values, where all six showed a more statistically consistent increase in Sudan containing treatments. Variables Net Energy of maintenance, Net Energy of gain as Mcal kg<sup>-1</sup> of dry matter were high in treatment 3, on the contrary, the Net Energy of lactation was high for corn silage alone. In this Table can be seen that with the addition of molasses to Sudan silage, there was as slight increase for most of variables when compared with treatments containing only Sudan silage. The lowest Digestible Energy was in this treatment again. Surprisingly corn silage alone had the best results for TDN, showing that it can be in some cases a good food without the addition of other ingredients when feeding cattle. Results in this Table show that corn silage itself or combined with different proportions of bovine feces is a better food for cattle compared with treatments containing Sudan silage even with the addition of molasses, although this ingredient slightly improved some variables. Nutritional value of silaged products is estimated by analyzing its chemical composition (Bogdan, 1997). It is necessary to consider that when using animal feces added to different plant forages easily fermentable there is an increase in crude protein but also in ashes and ADF contents, raising its buffer capacity which has a negative effect over fermentation (Al-Rokayan *et al.*, 1998; Rasool *et al.*, 1998; Fontenot y Jurubescu, 1980). The addition of ingredients easily fermentable such as molasses (4–6%) helps to increase fermentation of products during silage process. This results agree with those found by Tjandraatmadja *et al.* (1994) which evaluated in laboratory conditions plastic vacuum sealed bags containing 500 g of silage that were maintained in dark and controlled environment conditions the effect of adding 4 to 8% of molasses to panicum (*Panicum maximum* cv. Hamil), Pangola grass (*Digitaria decumbens*) and setaria (*Setaria sphacelata* cv. Kazungula) silages, concluding that even the lowest doses (4%) was adequate for the preservation of this three grass silage.

**Table 3.** Energetic composition of treatments.

Treatment	DE	NE <sub>m</sub>	NE <sub>g</sub>	NE <sub>l</sub>	TDN (%)
1	12.48 <sup>a</sup>	1.51 <sup>b</sup>	0.95 <sup>b</sup>	1.28 <sup>a</sup>	57.24 <sup>a</sup>
2	12.28 <sup>b</sup>	1.47 <sup>b</sup>	0.90 <sup>b</sup>	1.12 <sup>b</sup>	50.76 <sup>b</sup>
3	12.25 <sup>b</sup>	1.67 <sup>a</sup>	1.13 <sup>a</sup>	1.09 <sup>b</sup>	49.61 <sup>b</sup>
4	11.84 <sup>c</sup>	0.99 <sup>d</sup>	0.34 <sup>d</sup>	0.76 <sup>c</sup>	35.89 <sup>c</sup>
5	11.80 <sup>c</sup>	1.25 <sup>c</sup>	0.64 <sup>c</sup>	0.73 <sup>c</sup>	34.69 <sup>c</sup>
6	12.17 <sup>b</sup>	1.27 <sup>c</sup>	0.67 <sup>c</sup>	1.03 <sup>b</sup>	47.15 <sup>b</sup>
CV*	0.30	1.75	3.66	2.98	2.66

\*Coefficient of variation

DE = Digestible Energy (Mcal kg<sup>-1</sup> DM)

NE<sub>m</sub> = Net Energy of maintenance (Mcal kg<sup>-1</sup> DM)

NE<sub>g</sub> = Net Energy of gain (Mcal kg<sup>-1</sup> DM)

NE<sub>l</sub> = Net Energy of lactation (Mcal kg<sup>-1</sup> DM)

TDN (%) = Total Digestible Nutrients

## CONCLUSIONS

In general there was a great variation among treatments although in most cases those with corn silage had better results and some times similar to treatment of Sudan silage added with molasses. Corn itself gave good values for variables such as ADF and NDF. The addition of bovine feces increased Crude Protein and NEm and NEg but at the same time the ADF. Sudan itself was no good for most of variables but a better result occurred when bovine feces or molasses were added, improving the energetic value.

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## NUTRITIONAL CHARACTERIZATION OF FORAGE TREES FOR RUMINANTS FEEDING: IDENTIFICATION, INTAKE PREFERENCE AND TREE DENSITY (ADVANCES)

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### SUMMARY

The objective was to identify; determine intake preference and tree density of six of the most important forage species of trees present in Culiacán, Sinaloa, Mexico with potential to ruminant feeding. The species selected were those the cattle regularly eats in extensive pasturing conditions, among them are mauto (*Lysiloma divaricata*), vinolo (*Acacia cochliacantha*), ebano (*Caesalpinia sclerocarpa*), amapa (*Tabebuia* spp.) guacima (*Guazuma ulmifolia*) and palo pinto (*Pithecellobium mangense*). *Acacia cochliacantha* presented the highest tree density and intake preference, although *Pithecellobium mangense* had low tree density, showed a second place for intake preference. The other species were considered of intermediate importance.

**Keywords:** forage trees, intake preference, tree density, ruminants feeding.

### INTRODUCTION

Cattle activity in the agriculture based on rain regime in the central part of Sinaloa State has topographic problems, scarce tree covering and serious erosion problems, besides the incorrect utilization of new technologies such as ploughing the land the use of products to control weeds and pests. As a result, ruminant feeding is bad because of the low quality of forages (mainly Graminae) commonly used during the long period without rain which often last from seven to eight months a year and the reduce diversity of foods available, most of them of poor quality. The wrong cattle management practices utilized for many years have affected forage productivity and increased erosion. In this State most of time different kind of grain species are cultivated, being a great part annual and not always tolerant to dryness, partly because of the characteristics of radical systems, compare to the one some trees found in this area, many of them leguminous from which cows feed and that help to move nutrients from lower to upper strata of the soil (Araya *et al.*, 1994). When using a forage plant, mainly if it is not native, an integral study must be made, including its vegetative and reproductive development, radical system and nutritional value because its presence may change the structure, bromatologic composition, number and

characteristics of flora and soil. (Minson, 1990). These changes affect the forage utilization during the growing and flowering season, besides the weather conditions could also change forage quality (Van Soest, 1982; Jung, 1989) and these conditions are extreme in Sinaloa where temperature have raised up to 50°C during the last few years. Given that bromatologic composition is highly dependent on the species and conditions where it grows, it is possible to know almost certainly chemical season variations of forest trees products (Buxton y Fales, 1994), because will be possible to determine nutrient quality and therefore its management program to optimize their use. For example, the high crude protein content in forage trees may supplement cattle rations, decreasing the level of commercial protein concentrates, therefore lowering the cost of ruminants feeding. Barajas *et al.* (1992) began in the South of Sinaloa the studies of *in situ* degradation of some dependent of rain grasses (known as “temporaleros” grasses). In this State, since 1998 researches of the *in situ* digestibility of rations components or complete rations began at the Faculty of Agronomy, Universidad Autonoma de Sinaloa, Mexico from which some results have improved its knowledge and use by cattle owners (Guerra *et al.*, 1999; Guerra *et al.*, 2005). This has continued and recently began the study of lowland forages trees which are important for cattle in some areas during the dry season, for this reason it is important to begin and continue this research.

## OBJECTIVES

The objectives were to know the main tree species use as forage, characterize and determine its ruminal utilization and potential intake and possible addition to rations or partial substitution. With this long term research we also hope to give information to small cattlemen of “temporaleras” areas to improve their cattle feeding, weight gain and consequent productivity and also income through the use of leaves and other parts of forage trees found in central Sinaloa State, finally reducing the areas occupied by foreign grasses.

## MATERIAL AND METHODS

This research takes place in the central area of Sinaloa, Mexico, 107° 23' and 24° 55' North and about 88 m above sea level and means temperature of 25.1°C, being the minimum mean during January (19.3°C) and the maximum in July (30.3°C). The climate is defined as BS1 (h') w (e), warm semidry and extreme, with an annual rain average of 724.4 mm, being the maximum rain during August and the lowest in January (Köppen modified by García, 1987). In order to identify the forage trees eaten by ruminant cattle, three ways were utilized, a) a poll for cattlemen, regarding tree or bush species eaten by cattle, b) direct observation of cattle feeding at the lowland losing leaves forest in central Sinaloa to determine feeding frequency and species preference and c) by reading scientific information about the species already reported for other sites. According to Scheaffer y Mendenhall (1987) a randomized stratified sampling was used to identify *in situ* representative trees species for their later identification by specialists. Monthly samplings were taken using the sampling technique proposed by Shinozaki *et al.* (1964) which includes taking, identifying and weighting leaves and pods of the chosen trees. Samples were oven dried to constant weight (about 48 h) at 60°C. The optimum sampling size *per* species used was according to Scheaffer and Mendenhall, (1987) to estimate dry matter production. After drying, samples were finely grounded (Willey # 4) passed through a 1mm mesh, and put in glass tars for later



analysis of variables Apparent Dry Matter (at 60°C for about 48 h), Residual Dry Matter at 105°C for about 24 h, Crude Protein (Kjeldhal method), Ashes (AOAC, 1975), Neutral Detergent Fiber and Acid Detergent Fiber (Goering y Van Soest, 1970), Hemicelullose, Cell Content, Organic Matter and the Energetic Characterization were also evaluated (Undersander *et al.*, 1993), besides Green Matter Production (kg ha<sup>-1</sup>), Bromatologic Composition, Leave Area, Potential Intake and Ruminal Degradation of Green and Dry Matter and Protein Content. Analyses were performed at the Nutrition and Animal Bromatology Laboratory at the Facultad de Agronomía of the Universidad Autonoma de Sinaloa. The *in situ* degradability of Green Matter was carried out at la Posta Zootecnica of the Faculty of Agronomy, by using four Cebu cattle males 130 kg weight fistulated and with a rumen cannula to which an adaptation diet was given for ten days, giving 1.5 kg of commercial concentrate and alfalfa *ad libitum*. The *in situ* degradability was determined by using nylon bags, five replicates by plant species and by sampling month. Bags were taken out of animals at intervals of 0, 12, 24, 36 and 48 h (Orskov *et al.*, 1980; Orskov y Mc Donald, 1979). From the Neutral Detergent Fiber the Dry Matter Potential Intake and Protein Content were estimated (Pioneer, 1990; Schroeder, 1996; Thiex, 2001). Analysis of variance and later media compassion (Tukey  $\leq 0.5$ ) were made for variables evaluated (SAS version 9.2, 2004) using a randomize complete block design.

## RESULTS AND DISCUSION

Preliminary results of this research are here presented, but continue and probably will last two or three more years. The most important forage plants were determined through the polls applied to the most experienced cattle owners and the direct observation of animals feeding on them. Species identified as eaten by ruminants were Mauto (*Lysiloma* sp), Vinolo (*Acacia* sp), Ébano (*Lysiloma* sp), Amapa (*Tabebuia* sp), Guazima (*Guazuma* sp) and Palo pinto (*Pithecellobium mangense*). Seven monthly samplings were made, each at the end of every month, from May through November 2006. Because of changes of structure and morphological and composition of trees it is necessary to perform a long term research including climate variables (Minson, 1990). Additional information related to different uses of forage species by people and cattle is presented in Table 1. There and as a result of *in situ* observations we conclude that Vinolo (*Acacia* sp) is the main forage species followed by Palo Pinto (*Pithecellobium mangense*), Guazima (*Guazuma* sp), Ébano (*Lysiloma* sp), Mauto (*Lysiloma* sp) and Amapa (*Tabebuia* sp). For density, it was found that the previous order slightly changed, although being Vinolo (*Acacia* sp) again the first, ad then Mauto (*Lysiloma* sp) Guazima (*Guazuma* sp), Amapa, Palo Pinto (*Pithecellobium mangense*) and finally Ébano (*Lysiloma* sp). Given that in general results of bromatologic composition are highly dependent on species studied, soil, climate and other conditions were they grow, it is possible to know almost certainly the season variation in its chemical composition, what may help to determinate nutritive quality and the manage programs to improve this quality and availability for cattle feeding. One advantage of high crude protein contents of forage trees would help to add or lower the proportion of commercial concentrates, decreasing its cost.

**Table 1.** Characteristics and utilization and density of the most important forage trees found in the studied area in Culiacán, Sinaloa, México.

Collected material	Common name	Scientific name	Type of plant	Use	Intake	Density (per sampling area)
Leave and pods	Vinolo	<i>Acacia farnesiana</i> <i>Acacia cochliacantha</i> Humb. & Bonpl. ex Willd.	Tree	Lumber	1	1
Leave, pods and fruits	Amapa	<i>Tabebuia palmeri</i>	Tree	Furniture, lumber	5	4
Leave, pods and fruits	Mauto	<i>Lysiloma divaricata</i>	Tree	Fence	5	2
Leave and pods	Palo pinto	–	Tree	Fence lumber	2	5
Leave, pods and fruits	Guasima	<i>Guazuma ulmifolia</i>	Tree	Medicinal	3	3
Leave, pods and fruits	Ébano	<i>Lysiloma</i> sp	Tree	Lumber	4	6

## CONCLUSIONS

Up to date the advances may permit to state that Vinolo trees is the most consumed forage tree by cattle in the central area of Sinaloa, having at the same time the greatest density per hectare. Although Palo pinto was the second most preferred specie, its importance according to tree density was very low. The rest of species showed intermediate importance. It is necessary to end the analysis of data collected and continue with similar and extended experiments the next years.

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## MILK PRODUCTION IN SWEDISH PRIMIPAROUS DAIRY COWS ASSOCIATED WITH CALFHOOD REARING AND HEALTH

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### SUMMARY

Associations of first-lactation milk production with calthood rearing conditions and health were studied in 2,060 dairy heifers in 107 Swedish herds. Milk production at first test-day after calving (median 27.1 range 7.9–48.0 kg) and during first 305 days of lactation (median 8006; range 3,764–12,136 kg) were modelled. High age at calving was associated with higher production, indicating that high prepubertal weight gain can be compatible with high milk production. Calthood diarrhoea and fatness at 1<sup>st</sup> service was associated with a low production. The results indicate that accustoming heifers to large amounts of concentrates before calving is beneficial.

**Keywords:** dairy cattle, diarrhoea, management, milk production, prepubertal weight gain, rearing, replacement heifer

### INTRODUCTION

Feeding and management of the lactating cow influence her milk production. A high milk yield has also been associated with a successful rearing of the replacement heifer. First-lactation milk production is affected by body weight at calving (Carson et al., 2002), and by age at calving (e.g. Moore et al., 1991). In several studies, high feeding regimes and hence high daily weight gains after sexual maturity and during pregnancy have resulted in higher body weights at calving and increased milk production (Little and Harrison, 1981; Foldager and Sejrsen, 1991). However, at an early calving, accelerated postpubertal growth was found to be associated with lower first-lactation milk production (Hoffman et al., 1996) and no effect on mammary development and milk yield was reported by Sejrsen et al. (1982). There is substantial evidence of a negative effect of high weight gains during the period of allometric mammary growth, i. e. from approximately 90 to 300 kg of body weight (Sejrsen et al., 1982). However, contradicting results have been reported by e.g. Stelwagen and Grieve (1992) and Pirlo et al. (1997), possibly be due to different feed intensities, protein levels, periods of study, and ages at calving. Further studies using different types of data are needed to shed light on the complexities of these relationships.

Information on effects of health disturbances during the rearing period on subsequent milk production is surprisingly scarce. Diseases early in life have been associated with increased risks for morbidity later during the rearing period (e.g. Svensson et al., 2006), and long-term effects of calf morbidity on survival and age at calving have been reported (van der Fels-Klerx et al., 2002). Results from institutional herds may not be applicable to commercial production because they differ in management, veterinary service, and priorities. Warnick et al. (1995) studied commercial dairy farms, but failed to detect an association between calf morbidity and subsequent first 305-d

and second test-day milk production. Data comprised only 728 heifers from 25 herds and were based on owner-diagnosed calf diseases. Svensson et al. (2003) reported that only half the cases of pneumonia diagnosed by project veterinarian at bimonthly visits were recognized by farmers. Owner recording is hence likely to underestimate the true disease incidence and thus potentially biases the results.

The objective of the present observational study was to investigate the associations of housing, management and farmer- and veterinary-diagnosed clinical disease of dairy calves and replacement heifers on their subsequent first-lactation milk production.

## MATERIAL AND METHODS

One hundred and twenty-two dairy farms with 28 to 94 cows in the southwest of Sweden and enrolled in the official milk-recording scheme (Andersson, 1988) were selected based on their housing system for calves and replacement heifers (Lundborg et al., 2003). In 1998, herd sizes in the range of 28 to 94 cows represented 56% of all Swedish dairy herds. In the selected farms, a cohort of all heifer calves born in 1998 ( $n=3,081$ ) was studied from birth to first calving, or until removal from the study. In total, 179 (5.8%) of the animals died, 267 (8.7%) were culled, and 259 (8.4%) were sold before calving, and 250 (8.1%) were lost to follow-up. Fifty-eight cows (1.9%) lacked production data and 8 cows (0.3%) were excluded due to abortions. All of the remaining 2,060 animals from 107 herds were included (1,029 Swedish Reds, 999 Swedish Holsteins and 40 crossbreeds or animals of other breeds).

Calves were kept in single pens, or in group pens bedded with straw or sawdust until weaning, and then in group pens with slatted floors or deep litter. However, especially heifers on slats were subsequently transferred to similar housing systems as for the lactating cows in the herds, mainly tie-stalls. From breeding to calving, 4% of the heifers were kept in cubicles and 45% in other systems, mainly tethered. At calving, increases in concentrates started  $\geq 3$  weeks prepartum in most animals (73%). Grazing was generally practised between May and October; 9% of the heifers were not grazed.

Farmers and project veterinary surgeons, visiting the farms bimonthly to make a brief physical examination of the calves, recorded diseases prepartum. The veterinarians recorded information about building type, housing system, stocking rate, age distribution and indoor feed rations offered, and weighed the feed. They also measured the indoor air ammonia concentration, temperature and relative humidity where the animals were reared. For each calf, the farmers were requested to record the breed, the place and time of birth, whether or not the calving had been supervised, the time from birth to first observed ingestion of colostrum, the main method of feeding the first two meals of colostrum to the calf and the main source of the first two meals of colostrum fed to the calf. The farmers were also requested to measure the heart girth of the animals at birth, at weaning, at first service, at turn-outs to pasture and housings in their first and second grazing periods (or if not grazed during corresponding autumns), and at calving. The heart girths were transformed to live weights and corresponding daily weight gains. Body condition was scored by AI technicians at first insemination. Information on monthly milk production and SCC was obtained from the official milk-recording scheme. Milk production from the day after first calving until 305 d of lactation or culling (305-d milk production) was calculated.

Associations of the morbidity of the animals and their housing, feeding, and management before calving with first-lactation milk production were investigated by a linear mixed model using the MIXED procedure in SAS for Windows software package, version 9 (SAS Institute

Inc., Cary, NC, USA). Two continuous outcome traits expressed in kg of energy-corrected milk (ECM) were modelled: milk yield at first test-day before 81 d in milk (ECM1) and 305-d milk production (ECM305). In the model of ECM1, 2,059 observations from 107 herds were included. The analysis of ECM305 used 1,562 observations from 105 herds. Of the excluded records, the majority lacked production data from one or more test milkings.

A total of 67 independent cow- and herd-level variables, representing housing, management, feeding, growth, body condition and health during rearing were considered in the models. Among these variables, 12 were justified by hypotheses and the remaining were possible confounders. Calving weight was considered as a predictor but not included in final models because, based on initial modelling results, it was judged to be an intervening variable (Dohoo et al., 2003). Continuous variables were categorized. When biologically relevant categories were lacking, quartiles were used as cut-off points. Initially, univariable analyses were carried out, testing the predictors (one at a time) possibly associated with each trait (with a random-intercept effect of herd in model) and selecting those significant at Type 3  $P_F \leq 0.30$ . Based on previous knowledge and results from the described initial selection, confounding variables representing calving year, calving season, housing system after calving and breed were forced into all models henceforth.

Including the random-intercept effect of herd, remaining selected independent variables were tested once again (one at a time), this time retaining for further analyses only those significant at  $P_F \leq 0.20$  and denoting them eligible predictors (of one or both traits). These were type of confinement at birth; housing system from birth to 90 d of age; housing system from birth to first service; diarrhoea before 91 d of age; other disease before 91 d of age; respiratory disease before 91 d of age; daily weight gain from weaning to first service; body condition score at first service; age at calving; amount of concentrates fed 2 months before calving; amount of concentrates fed at calving; increase in concentrate ration 2 months before calving; calving weight; and number of cows in herd in 1998. To utilize as many observations as possible, missing values of continuous cow-level predictors were imputed as the mean value in that particular herd (550 records of calving weight, 443 of daily weight gain from weaning to first service, and 95 of daily weight gain until weaning). The final models were built by a manual stepwise procedure, starting with full models, continuing until all fixed effects were significant at Type 3  $P_F \leq 0.01$ . Thereafter, all first-order interactions were tested through a similar backward elimination and retained when  $P_F \leq 0.01$ ; no significant interactions were found. Random-intercept and random-slope terms at the herd level for all fixed effects in the model were tested for inclusion, retaining those significant in a likelihood-ratio test at  $P \leq 0.05$ .

## RESULTS

ECM1 ranged from 7.9 to 48.0 (median 27.1; inter-quartile range [IQR] =23.6–30.5) kg and ECM305 ranged from 3,764 to 12,136 (median 8,006; IQR=7187–8829) kg. Of the total variation in ECM1 and ECM305, 18% and 38%, respectively, resided at the herd level, and the remaining variation at the cow level (estimated from empty variance-component models, without fixed effects). Median age and weight at first service was 531 (IQR=488–605) days and 387 (IQR=357–430) kg, respectively. Median growth rate from weaning to 6–9 months was 726 (IQR=619–824; min.–max. =90–1253) g/day, and from 6–9 months until first service 625 (IQR=539–727; min.–max. =241–1254) g/day.

Higher ages at calving resulted in successively higher production; calving at >930 d gave 2.10 kg higher ECM1 and 975 kg higher ECM305 than calving at  $\leq 783$  d. Likewise, production

increased successively with higher daily weight gains from weaning to first service; at >738 g, cows had 1.7 kg higher ECM1 and 539 kg higher ECM305 than at ≤598 g. Calving during May to September resulted in a 1.5 kg higher ECM1 and a 166 kg higher ECM305 than calving during the rest of the year. Swedish Holsteins had a 0.8 kg higher ECM1 and a 184 kg higher ECM305 than Swedish Reds, and cows housed in short-stalls had a 2.6 kg higher ECM1 and a 680 kg higher ECM305 than those in cubicles. In addition, cows that had contracted mild diarrhoea during their first 3 mo of life had 344 kg lower ECM305 than those without diarrhoea, a body-condition score ≥3.2 at first service resulted in 256 to 337 kg lower ECM305 than a score ≤2.9, and a large increase in concentrate feeding during the last two months before calving was associated with a high production (at >13.2 kg increase, 876 kg higher ECM305 than at ≤9.5 kg increase). Furthermore, a SCC >1 million cells/ml at first test-day was associated with 1.5 kg less milk on the same day.

Most cases of diarrhoea before 91 d of age (67%) were mild and diseased calves recovered within short.

## DISCUSSION

There is convincing evidence for a higher production with increasing age at calving (e.g. Ettema and Santos, 2004), presumably due to a lower energy requirement for growth in older animals, which is supported further by our results.

Daily weight gains from weaning to first service exceeding 738 g/day (fourth quartile) were associated with the highest milk production, with successively higher production levels at increasing weight gains. High daily prepubertal weight gains have been associated with impaired mammary development (Sejrsen et al., 1982) and decreased subsequent first-lactation milk production (Foldager and Sejrsen, 1991). However, Hohenboken et al. (1995) and Zanton and Heinrichs (2005) found curvilinear relationships with positive slopes for daily prepubertal weight gains below approximately 650 and 800 g/day, respectively, and negative slopes for higher weight gains. The most critical period for nutritional influence on mammary gland growth is likely to be before puberty, at 3 to 9 months of age (Waldo et al., 1989). In our study, start of puberty was not recorded, but heart girth was measured either at 6 to 9 months of age or at corresponding time-points, and at first service. Although weight gain from weaning to first service included also some time after puberty, it was probably an acceptable estimate of prepubertal growth. According to Foldager and Sejrsen (1991), the negative influence of a high feed intake during part of the critical period cannot be compensated by subsequently reducing feed intake, even though the overall mean prepubertal growth rate is acceptable. Nevertheless, in this study, growth rates from weaning to 6–9 months exceeded those from 6–9 months to first service. Our data therefore indicate that high prepubertal weight gains are compatible with a high first-lactation milk production under practical Swedish conditions. Similarly, Pirlo et al (1997) reported that Italian Friesian heifers tolerated an average daily gain of approximately 800 g from 100 to 300 kg of body weight without any detrimental effect on future milk production. Based on path analysis, we judged calving weight to intervene in the causal pathway between prepubertal growth and milk production; we found no direct effect of prepubertal weight gain on first-lactation milk production. Controlling for diet, Silva et al. (2002) found that heifers that grew faster did not have impaired mammary development. They suggested that increased body fatness was a better predictor of impaired mammary development and found that body condition score at breeding was

negatively correlated with milk yield. This is supported by the fact heifers which were over conditioned at first service had a lowered milk production in the present study.

Higher increases in concentrate feeding during the last two months before calving were associated with a higher milk production compared to moderate increases. The effects of an adaptation to lactation feeding ration starting 3 weeks before calving was evaluated in multiparous cows by Olsson et al. (1998), who found little effect on subsequent milk production. However, it resulted in significantly higher yields in the first month postpartum. Mäntysaari et al. (1999) reported that a high feeding intensity during the last trimester was associated with higher milk yield, but found no effect of feeding intensity in early gestation.

To our knowledge, this is the first report of an association between calfhood morbidity and first-lactation milk production. Previous studies have included far less animals and hence provided lower statistical power. In the study by Warnick et al. (1995), calculations revealed a power of  $\geq 0.70$  to detect a reduction of 500 kg in 305-d milk yield using 728 animals from 25 commercial herds. Diagnoses were made by farmers. The present study, supplementing farmer-diagnoses with veterinary examinations every second month, detected a reduction of 344 kg per cow, corresponding to losses of approximately 100 € per cow. In the present material, most cases of diarrhoea were mild and diseased calves recovered within short.

## CONCLUSIONS

Our study confirms previous findings that an increased age at calving is associated with a higher milk production during first lactation, and provides further evidence that a high prepubertal weight gain can be compatible with high milk production and that body fatness at first insemination is associated with a reduced first-lactation production. Furthermore, they indicate that there might be benefits, from a production perspective, of accustoming heifers to large amounts of concentrates before calving. The study suggests that calf-hood diarrhoea is associated with a lowered milk production in the first lactation.

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## THEILERIOSIS AND BABESIOSIS IN CATTLE: HAEMOGRAM AND SOME BIOCHEMICAL PARAMETERS

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### ABSTRACT

A total number of 43 of field cases of cattle of both sexes were clinically and laboratory investigated in this study. 15 cattle of both sexes out of this number were found clinically healthy free from both internal, external and blood parasites. Clinical and laboratory examination revealed that about 20 animals were found suffering from theileriosis and 8 animals were found suffering from babesiosis.

Blood smears were prepared directly from ear vein for all animals and lymph smears were prepared from suspected cases of theileriosis. The blood smears were used for differential leucocytic count, while lymph smears were used for observation of Koch's blue bodies. Two blood samples from both clinically healthy and diseased cattle were collected from jugular vein, one with anticoagulant for examination of haematological picture and the other without anticoagulant for separation of serum which used for biochemical analysis.

Clinical examination revealed enlargement of superficial lymph nodes, fever, congested mucous membranes, corneal opacity and emaciation were found in cases of theileriosis, while fever, paleness of mucous membranes and brown coffee urine were common clinical findings in cases of babesiosis. Haematological findings revealed that cattle suffered from theileriosis showed normocytic hypochromic anemia, while those suffered from babesiosis showed normocytic normochromic anemia. Biochemical findings revealed that cattle infected with theileriosis and babesiosis showed decreased serum levels of albumin and total proteins with increased serum globulins. The serum level of glucose was significantly decreased in cattle suffered from theileriosis and babesiosis. Serum level of aspartate aminotransferase (AST) showed significant increase in both theileriosis and babesiosis, while the Serum level of alanine aminotransferase (ALT) was significantly increased only in case of babesiosis. Serum level of gamma glutamyltransferase (GGT) was significantly increased in both theileriosis and babesiosis. Cattle infected with theileriosis showed significant decrease in serum level of iron only, while those suffered from babesiosis showed significant increase of both iron and copper serum levels. The serum level of Total iron binding capacity was significantly decreased in theileriosis.

We can conclude from our study that theileriosis and babesiosis are associated with impairment and alteration of liver function.

**Keywords:** cattle; theileriosis; babesiosis; clinical findings; haemogram; biochemical parameters

## INTRODUCTION

Theileriosis and babesiosis are considered the important blood parasites of cattle which caused by *Theileria annulata* and *Babesia bovis* respectively and they are still representing a serious problem especially in tropical and subtropical areas. The importance of theileriosis and babesiosis is due to severe economic losses and their effect on the immune status of the body (Urquhart et al., 1996).

The most marked clinical signs of theileriosis in cattle are enlargement of the lymph nodes in the area draining the site of tick attachment followed by fever, depression, anorexia and drop in milk production. In later stages, there may be nasal and ocular discharges, dyspnea, and generalized lymph node enlargement. Severe cases may be associated with diarrhea and dysentery (Radostits et al., 2000). Cows with theileriosis showed systemic changes, lateral recumbency (Stockham et al., 2000). The most clinical signs of babesiosis in cattle were fever, anorexia, dark brown urine (Yeruham et al., 2003).

Marked anemia, anisocytosis, pikiocytosis and Leucopenia were commonly observed in bovine theileriosis (Ceci et al. 1997).

Sharma et al. (2000) mentioned that haemoglobin (Hb), packed cell volume (PCV), differential leucocytic count (DLC), total leucocytic count (TLC) and total erythrocytic count (TEC) were significantly decreased in bovine babesiosis and this might be due to the intravascular haemolysis.

Non-significant decrease in levels of total proteins, albumin and glucose reported by Sandhu et al. (1998), however, marked decrease in total serum proteins, albumin, serum immunoglobulin and albumin-to-globulin ratio in *Theileria* infected crossbred calves observed by Singh et al. (2001).

Levels of total serum proteins and blood glucose were declined during the hemolytic phase of *Babesia* infected cattle (Fujinaga, 1981), while Pandey and Misra (1987) found the protein profile normal.

The goal of this work designed to study the effect of natural infection with theileriosis and babesiosis in cattle on the clinical animal health condition, haemogram and some biochemical parameters.

## MATERIALS AND METHODS

### I-Materials

#### A-Animals

A total number of 43 cattle aging from two to four years old were admitted to veterinary teaching hospital of faculty of veterinary medicine. The chief complaints of 28 animals were persistent fever and anorexia. The other animals were admitted for pregnancy diagnosis and appeared clinically healthy, therefore used as a control group.

#### B-Samples

Whole blood and lymph smear were obtained for microscopical examinations, while serum samples was obtained for biochemical analysis of ALT, AST, GGT, Total proteins, albumin, glucose, iron, copper and total iron binding capacity (TIBC).

## II -Adopted methods

### A-Clinical Examination

Clinical examination of all cattle was carried according to Rosenberger (1990).

### B- Hematological Examination

Haematological examination was done including red blood cells count (RBCs) and white blood cells count (WBCs), haemoglobin (Hb), and packed cell volume (PCV) manually (Coles, 1986). In addition, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated mathematically, while differential leucocytic count was determined by four field meander method (Kelly, 1984).

### C-Biochemical Assay

Biochemical serum analysis of total proteins, albumin, glucose, ALT, AST, GGT, Iron, Copper, and total iron binding capacity (TIBC) were estimated spectrophotometry using commercial chemical kits supplied by Randox.

### D-Statistical Analysis

The obtained data were statistically analyzed by means of computer based statistical program (Borenstein et al., 1997).

## RESULTS

### A-Clinical Findings

Clinical findings of cattle suffering from theileriosis and babesiosis are listed as in table (1).

**Table 1.** Main clinical findings in clinically healthy cattle and diseased ones:

Clinical Findings	Healthy cattle	Theileriosis	Babesiosis
Body condition	Good	Emaciated	Thin
Mucous membranes	Bright red, moistened no lesions and filled episcleral blood vessels	Congested with obvious Lacrimation and corneal opacity also evident.	Pale and empty episcleral blood vessels.
Temperature	38.7°C	40.6°C	40.1°C
Pulse	57 beats / minute	76 beats / minute.	89 beats / minute.
Respiration	21 respiratory cycle / minute	36 respiratory cycle / minute.	39 respiratory cycle / minute.
Lymph nodes	No swelling, movable, hotless, painless	Greatly swollen, painful and hot during palpation.	No swelling, movable, hotless
Urine	Light yellow	Straw yellow	Dark brown to coffee in color.

### B-Haematological Findings

The mean values of haemoglobin (Hb), red blood cells count (RBCs), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells count (WBCs) and differential leucocytic count are listed in table (2).

**Table 2.** Mean values<sup>#</sup> of haemogram in health cattle and diseased ones:

Parameters	Healthy cattle	Theileriosis	Babesiosis
<b>RBCs (T/l)</b>	7.67±0.43 5.69–11.60	6.07±0.19** 5.58–7.50	3.09±0.38*** 2.15–5.30
<b>Hb (g/l)</b>	118.93±3.07 95.00–150.00	87.60±7.18*** 59.00–130.00	45.37±0.98*** 40.00–50.00
<b>PCV (%)</b>	36.47±0.75 28.00–40.00	33.40±2.007 26.00–48.00	14.25±1.28*** 10.00–20.00
<b>MCV (fl)</b>	49.11±2.29 32.76–64.00	55.31±2.45 <sup>NS</sup> 43.70–65.00	49.46±4.57 22.64–64.93
<b>MCH (Pg)</b>	16.19±0.82 10.17–21.89	14.66±1.41 9.07–23.30	16.21±1.62 8.49–21.64
<b>MCHC (%)</b>	32.96±0.69 30.70–40.00	26.36±1.74*** 18.75–36.10	33.48±2.48 23.50–45.00
<b>WBCs (G/l)</b>	7.25±0.42 4.55–11.00	5.00±0.36*** 3.90–7.55	6.77±0.67 4.35–8.40
<b>Neutrophils (%)</b>	32.33±1.31 23.00–40.00	18.40±1.19*** 12.00–23.00	23.62±1.31*** 18.00–30.00
<b>Lymphocytes (%)</b>	61.53±1.49 52.00–72.00	70.60±1.11*** 64.00–76.00	68.75±1.38** 64.00–75.00
<b>Eosinophils (%)</b>	3.133±0.31 2.00–5.00	3.00±0.33 2.00–4.00	2.50±0.27 1.00–3.00
<b>Basophils (%)</b>	0.00±0.00 0.00–0.00	0.00±0.00 0.00–0.00	0.00±0.00 0.00–0.00
<b>Basophils (%)</b>	0.00–0.00 2.93±0.28	0.00–0.00 7.40±0.69***	0.00–0.00 5.12±0.35***
<b>Monocyte (%)</b>	1.00–5.00	5.00–12.00	4.00–7.00

# = Mean ± Standard error

NS=Non significant

\*\* = P < 0.01

\*\*\* = P < 0.001

### C-Biochemical Findings

The serum levels of total proteins, albumin, globulins, albumin-globulin ratio (A/G ratio), glucose, AST, ALT, GGT, iron, copper and total iron binding capacity (TIBC) are listed in table (3).

**Table 3.** Mean values<sup>#</sup> of some biochemical parameters in healthy cattle and diseased ones:

Parameters	Healthy cattle	Theileriosis	Babesiosis
<b>Total proteins (gm %)</b>	6.92±0.15	5.58±0.33***	5.82±0.28***
	6.00–8.00	4.14–7.50	4.46–7.10
<b>Albumin (gm %)</b>	3.64±0.0085	1.58±0.0093***	1.86±0.16***
	3.10–4.30	1.00–2.00	1.45–2.67
<b>Globulins (gm %)</b>	3.28±0.0098	4.00±0.30*	3.96±0.38*
	2.70–4.10	2.60–5.81	2.75–5.62
<b>A/G ratio</b>	1.13±0.003	0.41±0.004***	0.54±0.009***
	.90–1.37	0.25–0.59	0.26–0.91
<b>Glucose (mg %)</b>	71.31±3.48	54.99±4.09**	32.71±3.12***
	55.00–100.00	26.00–66.90	20.74–44.00
<b>AST (U/l)</b>	28.34±1.6	59.98±5.81***	130.26±10.62***
	18.00–41.00	31.34–93.19	93.19–171.70
<b>ALT (U/l)</b>	13.78±0.70	14.99±0.73	26.08±1.14***
	7.00–18.00	11.20–19.89	19.89–31.04
<b>GGT (U/l)</b>	9.91±0.97	13.01±0.66**	13.79±0.44**
	4.44–19.60	9.63–15.70	11.85–15.90
<b>Copper (µg/dl)</b>	98.34±2.15	97.69±2.03	273.50±16.66***
	86.96–107.04	92.17–104.35	222.10–320.00
<b>Iron (µg/dl)</b>	188.25±15.49	64.29±5.57***	245.95±15.45*
	138.00–273.68	54.40–85.30	210.04–298.56
<b>TIBC (µg/dl)</b>	506.78±30.33	198.62±18.21***	412.41±13.86
	334.5–671.00	175.79–271.01	372.42–453.63

# = Mean ± Standard error

\* = P < 0.05

\*\* = P < 0.01

\*\*\* = P < 0.001

## DISCUSSION

### A–Clinical findings

The observed clinical findings in cattle with theileriosis such as anorexia, corneal opacity, enlarged superficial lymph nodes. These findings were in agreement of Shehata et al. (1984), Sandhu et al. (1998) and Radostits et al. (2000). Anorexia could be attributed to persistent fever; moreover the enlargement of superficial lymph nodes could be explained by lymphoid hyperplasia in early stage of the disease. The corneal opacity was explained by Irvin and Mawmachi (1983) as a result of white blood cells infiltration.

The observed clinical findings in cattle with babesiosis such as fever, dark brown to coffee urine, pale mucous membranes with empty episcleral blood vessels with reduced appetite could be attributed to severe haemolytic process associated the presence of *Babesia sp.* inside the red blood cells. Fujinaga (1981) and Georgi et al. (1990) supported this view.

### **B–Haematological findings**

The normocytic hypochromic anemia observed in cattle with theileriosis (table 2) could be attributed to the toxic metabolites of *Theileria sp.* which have harmful effect on bone marrow as they interfere with the process of erythropoiesis. Persistent loss of blood caused by permanent blood sucking ticks could play a role as well. Boulter and Hall (2000) mentioned that Tumor necrosis factor- $\alpha$  (TNF-  $\alpha$ ) has been implicated in the pathogenesis of anemia in bovine theileriosis by suppressing haemopoietic progenitors.

Leucogram showed significant decrease ( $P<0.001$ ) in total leucocytic count and neutrophils while the lymphocytes and monocytes showed significant increase ( $P<0.001$ ) in comparison with healthy control ones. Such changes in Leucogram might be attributed to persistent harmful effects of toxic metabolites of *Theileria* on the haemopoietic organs especially bone marrow and their interference with the process of leucogenesis. Relative increase in numbers of lymphocytes and monocytes reflects compensatory mechanism as target cells in response to their invasion with *Theileria* protozoan. Similar results were observed in *Theileria* infected cattle by Lamiaa (1997).

Normocytic normochromic anemia observed in cattle with babesiosis which could be attributed to intravascular haemolysis of red blood cells. Pandey and Misra (1987) supported this view. Insignificant changes in total leucocytic count in total leucocytic count in cattle with babesiosis, while there was significant increase in lymphocytes and monocytes associated with significant decrease ( $P<0.001$ ) in neutrophils. This could be explained as the breakdown of red blood cells by *Babesia sp.* stimulates the phagocytic cells such as lymphocytes and monocytes to clean up the body from the toxic remnants of ruptured red blood cells. This is in agreement with both Guglielmone et al. (1996), who reported that *Babesia* infection lead to stimulation of body defense mechanism to produce antibodies against *Babesia* antigen, and Court et al. (2001), who mentioned that the significant increase in monocytes in primary *Babesia* infection could be attributed to their role as active mediators in the innate immune response.

### **C–Biochemical findings**

Theileriosis and babesiosis infected cattle showed significant increase in AST, GGT hypoproteinemia, hypoalbuminemia, and decreased A/G ratio. This may indicate the harmful effect of toxic metabolites of *Theileria sp.* and *Babesia sp.* on liver cells. These results were supported by Stockham et al. (2000) in *Theileria* infected cattle and Yeruham et al. (2003) in *Babesia* infected calf. The significant increase in serum globulins in both theileriosis and babesiosis could be attributed to the immune response against *Theileria* and *Babesia*. Both of Singh et al. (2001) and Fujinaga (1981) supported this view in theileriosis and babesiosis respectively. The observed hypoglycemia in both theileriosis and babesiosis could be attributed to persistent feverish condition associated theileriosis and babesiosis resulting in anorexia consequently hypoglycemia. This view supported by Sandhu et al. (1998) in *Theileria* infected cattle and Fujinaga (1981) in *Babesia* infected cattle. Serum level of iron was significantly decreased ( $P<0.001$ ), while copper showed insignificant change. The drop in serum level of iron may be due to anaemia which leads to excessive withdrawal of serum iron to be utilized for erythropoiesis. Omer et al. (2003) reported decrease serum iron and copper concentrations in cattle naturally infected with *Theileria annulata*. The increased serum levels of iron and copper in babesiosis could be attributed to haemolysis associated *Babesia* infection. Pandey and Misra (1987) adopted similar view where they explained the increase in serum iron and copper in *Babesia* infected cattle to intravascular haemolysis.

We can conclude that deteriorated body condition, fever, and anorexia are common clinical findings in cattle infected with theileriosis and babesiosis, however enlargement of lymph nodes and corneal opacity are associated clinical findings with theileriosis, while red brown to coffee

urine and pale mucous membranes are associated clinical findings with babesiosis. Normocytic hypochromic anaemia is associated with theileriosis, while normocytic normochromic anaemia is associated with babesiosis. Phagocytic cells lymphocytes and monocytes are commonly increased in *Theileria* and *Babesia* infected cattle. Theileriosis and babesiosis have harmful effect on the liver function in cattle.

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## EFFECTS OF GRAZING ON THE PERFORMANCE AND BEHAVIOUR OF BEEF BULLS

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### SUMMARY

We compared performance and behaviour of finishing Hereford bulls raised at pasture and in an uninsulated barn. Grazing led to leaner carcasses and improved the content of healthy fatty acids (e.g. CLA) in the meat, making the meat more compatible with consumer requirements. Differences in the time-budgets between the housing environments resulted mostly from the different feeding regimes and different space allowances. Stereotyped tongue-rolling was absent and there were no differences between the environments in time spent butting. This indicates that both housing environments were satisfactory in regard to the bulls' welfare. However, more synchronised behaviour in the pasture bulls indicates better opportunities for species-typical social behaviour at pasture.

**Keywords:** grazing, beef production, bulls, performance, fatty acids, behaviour

### INTRODUCTION

The use of pasture for finishing bulls is not widespread in Finland because this practise has been attributed to poor growth performance of the bulls (Nisula and Hakkola 1979). However, pasture is one of the most effective diets for elevating conjugated linoleic acid (CLA) and polyunsaturated fatty acids (PUFA) content of both milk (Kelly et al. 1998) and meat (French et al. 2000). The interest in CLA research is associated to its positive effects on human cancer, cardiovascular disease, diabetes, body composition, lipid metabolism, immune system, bone health and oxidation (Scollan et al. 2006). From an animal's point of view, grazing could be commendable due to increased possibilities for behavioural freedom of the animals, especially because ethical concern over intensive animal production has increased (Sørensen et al. 2001). We investigated how grazing affects the performance, fatty acid composition of the meat, and behaviour of finishing Hereford bulls.

### MATERIAL AND METHODS

The experiment was conducted at the North Ostrobothnia Research Station of MTT Agrifood Research Finland in Ruukki (64°44'N, 25°15'E). Twenty-nine Hereford bulls were used in the experiment. They were kept at pasture during their first summer 2004 and in an uninsulated barn

during the following winter. At the beginning of June 2005, the bulls (average age 14 months and weight 528 kg) were assigned to six groups of 4–5 animals. Three groups of the bulls were moved to perennial timothy pastures. Each pasture group was rotationally grazed six paddocks (0.34 ha per paddock) with animals being moved to a new paddock on average once a week. Three groups of the bulls were housed in partly bedded pens (6.4–8.0 m<sup>2</sup>/bull) in an uninsulated barn and fed grass silage *ad libitum*. Both pasture and barn bulls got barley 4.4 kg DM per animal per day. There was 0.7–0.9 m and 0.5 m feeding space per bull at the feeding trough in the barn and at pasture, respectively.

The behaviour of both barn and pasture bulls was observed directly for 24 hours in both June and July using instantaneous sampling method with a 6-min sampling interval. Observations of June and July were pooled for housing environments prior to analysis. The percentages of the observations spent on different behavioural patterns were tested with a linear mixed model. In the model, the housing environment was included as a fixed effect and the group in the housing environment as a random effect. If the residuals of the variables were not normally distributed, the variables ( $x$ ) were transformed with a formula  $\ln(x + 1)$ . Synchronisation of the lying and feeding behaviour was tested with  $\chi^2$ -test.

Grazing season extended 62 days (1.6.–1.8.2005) and after that both pasture and barn bulls were slaughtered. The live weight gain (LWG) was calculated as the difference between the means of initial and final live weights (LW). The carcasses were scored for conformation (scale from 1 to 15) and fat cover (scale from 1 to 5) using the EUROP quality classification. Fatty acid composition of the meat was measured from *Longissimus dorsi* muscle by gas chromatographic analysis (Metcalf and Schmitz 1961, Hara and Radin 1978). Animal performance data was subjected to analysis of variance using general linear models procedure.

## RESULTS AND DISCUSSION

Live weight data of the bulls before and during the grazing season are shown in Figure 1. There was no significant difference ( $P > 0.05$ ) in the LWG (average 1529 g/d) between the barn and pasture bulls during the grazing season. There were no significant effects ( $P > 0.05$ ) of housing environment on the carcass weight (average 337 kg) and carcass conformation score (6.5). The carcass fat score of the barn bulls was higher than that of the pasture bulls (2.9 vs. 3.3,  $P < 0.05$ ). Leaner carcasses of the pasture bulls probably resulted from locomotion in a large living space (see Table 2), and occasionally rather low sward herbage mass.

The proportion of *cis-9, trans-11* CLA, 18:2 *n-6* (linoleic acid) and 18:3 *n-3* ( $\alpha$ -linolenic acid) fatty acids in *Longissimus dorsi* muscle were higher in the pasture bulls than in the barn bulls (Table 1). In addition, compared to barn-housing, grazing increased proportion of 18:1 *n-7* fatty acid and decreased proportion of 14:1 *n-5* and 16:0 fatty acids. Also French et al. (2000) and Realini et al. (2004) have reported that grazing increases the CLA content of beef. However, according to Nuernberg et al. (2002) grazing has no effect on the CLA content of beef when grazing was compared to concentrate feeding in Simmental bulls and Holstein steers.

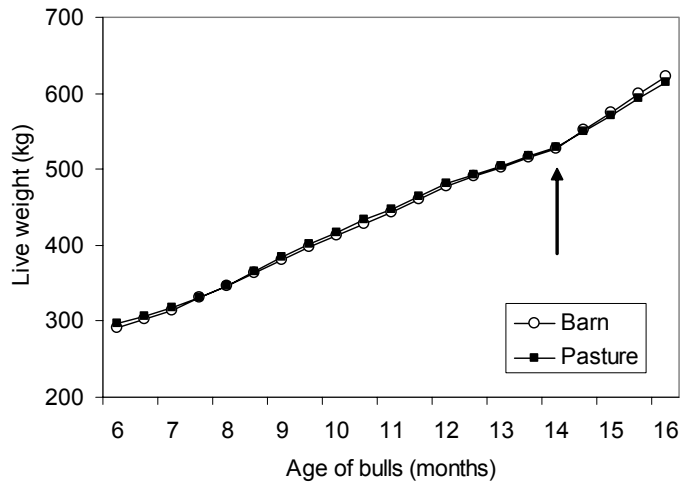
**Table 1.** Fatty acid profiles (g/kg of total fatty acids) (mean  $\pm$  SD) in Longissimus *dorsi* muscle of Hereford bulls housed in barn and at pasture.

Fatty acid	Barn	Pasture	Effect
14:0	22.2 $\pm$ 7.5	19.5 $\pm$ 4.2	
14:1 <i>n</i> -5	1.3 $\pm$ 1.9	0.1 $\pm$ 0.5	*
15:0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	
16:0	214.0 $\pm$ 16.1	201.1 $\pm$ 11.8	*
16:1 <i>n</i> -7	28.4 $\pm$ 7.4	25.3 $\pm$ 5.7	
17:0	9.4 $\pm$ 2.0	10.2 $\pm$ 2.0	
17:1	6.6 $\pm$ 0.7	6.6 $\pm$ 1.0	
18:0	179.3 $\pm$ 22.2	187.4 $\pm$ 22.0	
18:1 <i>n</i> -7	15.7 $\pm$ 1.5	18.0 $\pm$ 1.8	***
18:1 <i>n</i> -9	346.1 $\pm$ 20.1	340.6 $\pm$ 16.0	
18:2 <i>n</i> -6	68.8 $\pm$ 20.1	84.4 $\pm$ 16.7	*
18:2 <i>cis</i> -9, <i>trans</i> -11 CLA	2.8 $\pm$ 0.9	4.2 $\pm$ 1.5	**
18:3 <i>n</i> -3	15.3 $\pm$ 3.3	20.0 $\pm$ 3.7	**
20:0	1.1 $\pm$ 0.4	1.1 $\pm$ 0.6	
20:1 <i>n</i> -9	0.5 $\pm$ 0.4	0.6 $\pm$ 0.6	
20:2 <i>n</i> -6	2.5 $\pm$ 0.8	2.5 $\pm$ 0.8	
20:3	3.8 $\pm$ 1.4	3.9 $\pm$ 1.3	
20:4 <i>n</i> -6	27.8 $\pm$ 14.0	26.9 $\pm$ 7.7	
20:5 <i>n</i> -3	5.9 $\pm$ 3.9	7.5 $\pm$ 2.4	
22:5 <i>n</i> -3	10.5 $\pm$ 4.7	10.5 $\pm$ 2.8	
Unidentified fatty acids	37.8 $\pm$ 11.6	29.5 $\pm$ 9.8	*
SFA <sup>1</sup>	425.9 $\pm$ 42.8	419.4 $\pm$ 31.5	
MUFA <sup>2</sup>	398.9 $\pm$ 21.0	391.3 $\pm$ 21.4	
PUFA <sup>3</sup>	137.4 $\pm$ 4.5	159.8 $\pm$ 31.7	

<sup>1</sup> Saturated fatty acids, <sup>2</sup> Monounsaturated fatty acids, <sup>3</sup> Polyunsaturated fatty acids.

\* P<0.05; \*\* P<0.01; \*\*\* P<0.001.

The barn bulls ruminated more than the pasture bulls (Table 2). According to Kaustell et al. (1995), time spent ruminating and chewing increases in dairy cows as digestibility of silage decreases and fibre content increases. In our study, the neutral detergent fibre (NDF) content of silage that was offered to the barn bulls was higher (508 g/kg DM) and *in vitro* digestibility was lower (710 g/kg DM), than corresponding values of grazed grass (NDF 479 g/kg DM; *in vitro* digestibility 730 g/kg DM). This explains the higher ruminating time in barn bulls compared to the pasture bulls. The higher proportion of walking in the pasture bulls compared to the barn bulls was probably a natural consequence of the larger living area in the pasture. Walking during grazing was not taken into account in our study, and therefore the pasture bulls were actually moving even more than current results indicate. Increased energetic demand of locomotion may be partially responsible for the leaner carcasses of the pasture bulls compared to the barn bulls. Stereotyped behaviour such as tongue-rolling was not observed in either of the housing environment.



**Figure 1.** Live weight development of Hereford bulls housed in the barn and at pasture. The arrow indicates turnout to grazing of the pasture bulls.

**Table 2.** Percentage of observations (mean of observations  $\pm$  SD) spent on different behavioural patterns in bulls housed in barn and at pastures.

Behaviour	Barn	Pasture	Effect
Eating silage or barley at the feeding trough	11.5 $\pm$ 1.9	3.0 $\pm$ 0.8	***
Grazing	–	18.0 $\pm$ 2.7	–
Ruminating	33.9 $\pm$ 1.4	26.6 $\pm$ 3.3	**
Manipulating objects with mouth or tongue	0.23 $\pm$ 0.22	0.12 $\pm$ 0.17	
Drinking	0.70 $\pm$ 0.42	0.28 $\pm$ 0.31	
Walking excluding walking during grazing <sup>1</sup>	0.63 $\pm$ 0.29	3.2 $\pm$ 0.9	**
Self-grooming <sup>1</sup>	3.3 $\pm$ 1.7	1.8 $\pm$ 0.9	
Social licking	1.4 $\pm$ 0.5	0.60 $\pm$ 0.37	*
Butting <sup>1</sup>	2.2 $\pm$ 1.0	3.6 $\pm$ 2.1	
Lying inactive or resting	32.0 $\pm$ 3.9	28.5 $\pm$ 3.1	
Tongue-rolling	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	
Other behaviours e.g. idling in standing position	14.1 $\pm$ 3.1	14.6 $\pm$ 2.8	

<sup>1</sup> P-values are based on comparisons of estimated marginal means of  $\ln(x + 1)$  transformed variable.  
 \* P<0.05; \*\* P<0.01; \*\*\* P<0.001.

Lying behaviour was more synchronised in the pasture than in the barn bulls. All bulls within a group were observed to lie simultaneously more often at pasture (63.9% of lying observations, mean of June and July,  $\chi^2 = 150.7$ ,  $df = 1$ ,  $P < 0.001$ ) than in the barn (37.3%). Also cows lay simultaneously more often in pasture than inside cubicle house (O'Connell et al. 1989, Miller & Wood-Gush 1991). Mogensen et al. (1997) and Nielsen et al. (1997) have found that synchrony of lying decreases as space allowance decreases. Therefore, space allowance seems to have an important impact on the synchrony of lying. In our study, the pasture bulls had ample space,

whereas the barn bulls had only 3.2–4.0 m<sup>2</sup> bedded lying area per bull. This probably led the barn bulls to lie in less synchronised fashion than the pasture bulls.

Feeding behaviour was also more synchronised in the pasture than in the barn bulls. All bulls within the group were observed to eat silage or barley or graze simultaneously more often at pasture (16.8% of feeding observations, mean of June and July,  $\chi^2 = 67.5$ ,  $df = 1$ ,  $P < 0.001$ ) than in the barn (1.6%). The bulls ate alone more often in the barn (66.8% of feeding observations,  $\chi^2 = 114.1$ ,  $df = 1$ ,  $P < 0.001$ ) than ate or grazed at pasture (33.2%). Also Cozzi and Gottardo (2005) have found that pen-reared bulls with 95 cm feeding space per bull eat mostly alone or in pairs. Miller and Wood-Gush (1991) have suggested that the cause for unsynchronised behaviour in housed cattle is competition for resources that could lead to the animals feeding and resting at different times to avoid excessive aggression.

## CONCLUSIONS

Leaner carcasses of the pasture bulls probably resulted from locomotion in a large living area, and occasionally rather low sward herbage mass. Grazing improved the content of healthy fatty acids in the meat of the pasture bulls. Behavioural study revealed some differences in time-budgets between the housing environments, which probably resulted mostly from the different feeding regimes and different space allowances. Stereotyped tongue-rolling was absent in both environment and there were no differences between the environments in time spent butting. This indicates that both housing environments were satisfactory in regard to the bulls' welfare. However, more synchronised behaviour in the pasture bulls indicates better opportunities for species-typical social behaviour at pasture.

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## HOW BEHAVIOUR PREDICTS ACUTE ENDOTOXIN MASTITIS IN DAIRY COWS?

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### SUMMARY

There is a need to distinguish sick individuals automatically in large dairy barns but we lack of knowledge of which behavioural features change in disease outbreaks. To study this, we induced acute endotoxin mastitis in one quarter of six dairy cows and filmed their behaviour continuously during the placebo day and at the mastitis day. We found that the cows' resting behaviour changed rapidly after the onset of endotoxin mastitis. No differences were noted in the number of steps the cows took. Resting and rumination are promising behaviours to use as indicators when the health status of a cow is changing and to be further tested for automatic disease detection.

### INTRODUCTION

Increasing herd sizes and rapid growth of labour costs in Europe have led to a demand for automation in livestock production (de Koning & Rodenburg, 2004). There is a strong trend that the number of cows per farm increases and at the same milk yield per cow increases due to the efficient breeding of animals. Furthermore, distinguishing sick animals in dairy herds becomes more demanding. An effective human-animal-technology relationship creates a basis for animal health and welfare, as well as for optimizing the use of new technological solutions (Kaihilahti et al. 2007). For the moment, the commercially used technologies for detecting deviations in milk quality have been used to describe the udder health. However, these parameters have not been precise enough to be able to detect early signs of illness.

Automatic behaviour detection would be a feasible method, as the changes in behaviour are used by veterinarians and care-takers in the diagnosis of disease (Broom, 1987). However, we lack of knowledge of which behavioural features change, and how, in different disease outbreaks.

To study this, we induced an acute endotoxin mastitis in one quarter of 6 dairy cows each in late lactation and filmed their behaviour continuously during the placebo day (-1 day before induction) and at the mastitis day (day 0, induction day). The behavioural features were compared to clinical findings and milk parameters.



## MATERIALS AND METHODS

The experimental procedures were approved by the Ethical committee for the use of Laboratory Animals at the University of Helsinki.

The experimental cows were of Finnish Ayrshire or Holstein-Friesian breeds. Five experimental cows were at their 1<sup>st</sup> lactation and one at her 2<sup>nd</sup> lactation. Cows were housed in a stanchion barn and fed high quality silage *ad libitum* and concentrates six times a day. The cows were milked twice a day at 5.30 a.m. and 4.30 p.m.

Before the experiment the health status of animals was examined and they were clinically healthy. All of the quarters were sampled for milk somatic cell counts (SCC) and bacteria during three consecutive days. All samples were free from bacteria, and the mean cell count of all four quarters was less than 250 000 cells/ml and respectively, for the studied and control quarters less than 100 000 cells/ml.

On the day -1 at 7 a.m. we infused 5ml of saline in the left front quarters. On the following day (day 0) we challenged the same quarter with 10 µg of *Escherichia coli* O55:B5 lipopolysaccharide (LPS) (Sigma®, Sigma-Aldrich, Inc., Missouri, USA) diluted in 5 ml of sterile saline. Right front quarter served as a control.

During the follow up of the inflammation, we took 8 milk samples from the study and control quarters; 0, 2, 4, 6, 8, 10, 12 and 24 hours before and after the induction. From milk samples we analysed several parameters, such as SCC, electric conductivity and N-acetyl-β-D-glucosaminidase (NAGase) activity. Clinical examination included determination of systemic signs, as rectal temperature (°C), heart rate (beats/min) and determination of local signs as palpation of the udder and visual estimation of milk appearance.

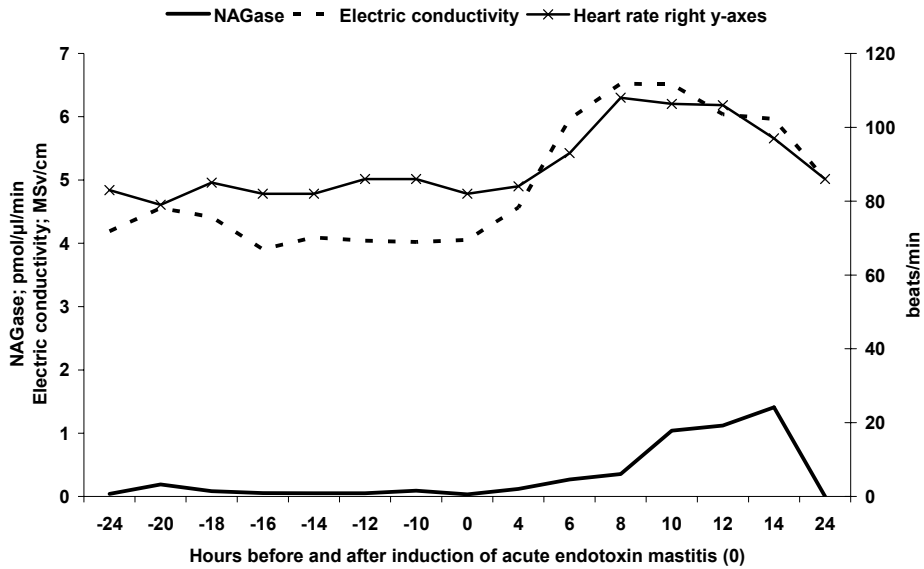
Bacterial samples were analyzed using conventional methods (Hogan et al., 1999). CMT was performed cow-side using a scale from 1 to 5 (Klastrup, 1975). Electrical conductivity was measured with a hand-held meter (Lutron CD-4301). SCC was analyzed with an electronic counter DCC (DeLaval International AB, Tumba, Sweden). Milk NAGase activity was measured with a fluorometric method (Mattila and Sandholm, 1986).

Cows were filmed continuously for 48 hours. From the videos we analysed mean hourly bout durations, frequencies and the total durations the cows spent resting or ruminating. In addition we counted the number of steps the cows took per hour.

We analysed the differences between days in behavioural and milk parameters and clinical findings with a mixed model, taking repeated observations into account. Fixed factors were hour and day, and an hour\*day interaction. Cow was a random factor.

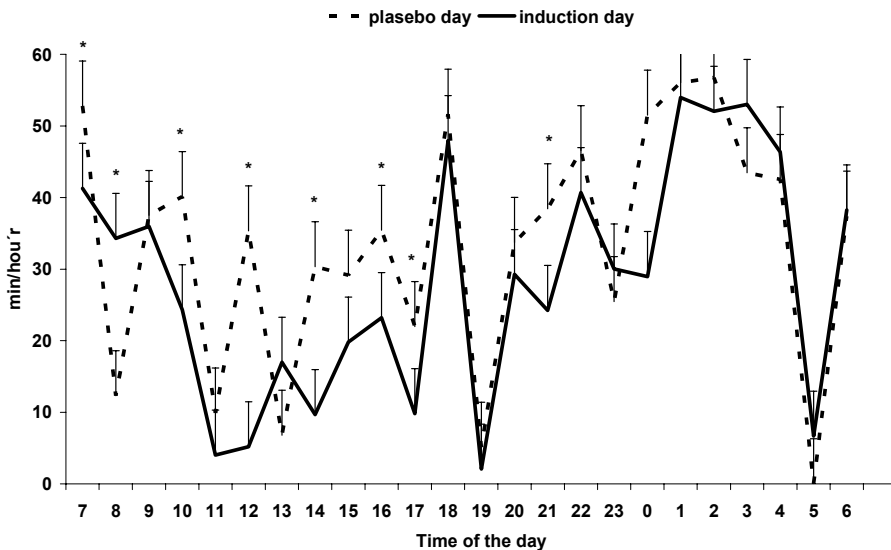
## RESULTS

All cows created both systemic and local signs after the LPS challenge. Heart rate, milk electric conductivity, SCC and NAGase activity started to increase 6 hours after induction and stayed high during the following 24 hours (Graph 1.). Body temperature started to rise 4 hours after induction, reaching the peak values 6 hours after induction and returned to normal 12 hours after induction (Graph 3).



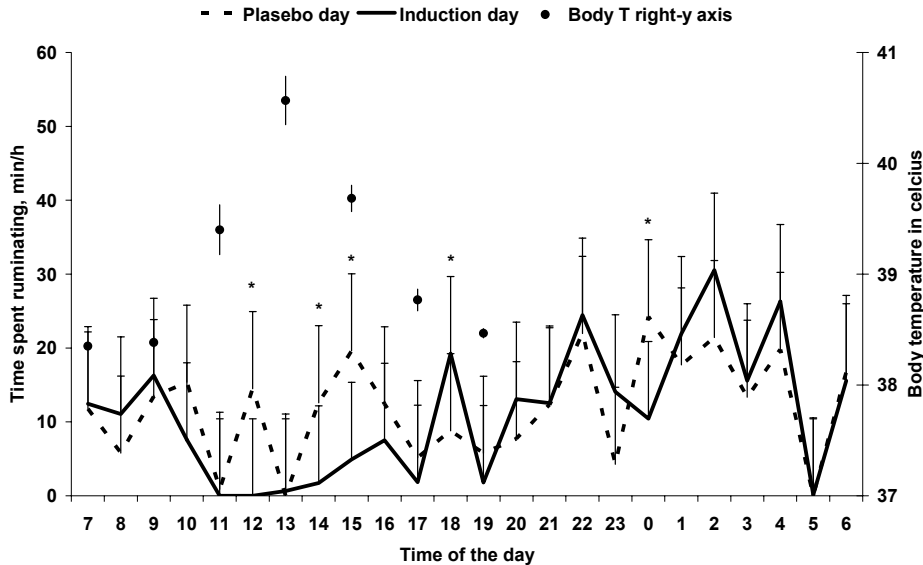
**Graph 1.** Mean heart rate and milk NAGase and electric conductivity 24 hours before and after induction of an acute endotoxin mastitis

Immediately after endotoxin induction, cows rested for longer, but afterwards, within the next 13 hours, the mean hourly resting time was shortened (Graph 2.).



**Graph 2.** The mean hourly duration spent lying down in dairy cows before and after the induction of an acute mastitis

And further, within 4 to 8 hours after induction, cows ruminated less than during the according hours at the control day (Graph 3). No differences were noted in the number of steps the cows took.



**Graph 3.** The mean body temperature and an hourly duration the dairy cows spent ruminating before and after the induction of an acute mastitis

## DISCUSSION

We showed here that the cows adapted to ongoing mastitis by changing their resting behaviour. When the infection proceeded, it affected also the behavioural signs; during two hours after the induction of an acute mastitis, cows were resting more than during the day before. We suggest this was due to the acute effect of cytokines. And further, that reduced activity and increased sleepiness are cows' strategies of energy conservation in order to allow the full development of a fever (Aubert 1997; Johnson 2002).

When the mastitis progressed and the clinical symptoms in the udder were more evident, the cows were resting less than the day before. We suggest that cows reduced their time spent lying down due to uncomfortable feeling and/or pain. Cows have a strong motivation to lie down (Jensen et al. 2005), but they prevent themselves from doing so, if the lying surface is uncomfortable enough (Haley et al. 2001). Sickness behaviour is the expression of a motivational state rather than the result of weakness (Aubert 1999; Johnson 2002). Thus, the pain-motivated behaviours of cows in this study overtook their sickness-motivated behaviours, such as lying down due to fever and/or weakness, similar to suggestions of Aubert (1997). The changes in behavior of the cows with an acute mastitis reflect changes in behavioral priorities (Johnson 2002).

Fever was reducing cows' rumination. This is also in line with the adaptive response of the sickness behaviour. The energetic cost of fever is rather high e.g. a metabolic increase of 13% is

necessary to raise body temperature by 1°C (Kluger 1991). This could contribute to the inhibition of energetically expensive behaviours, such as rumination.

The changes in the resting behaviour and rumination had disappeared after 17 hours from the induction of mastitis, although the milk appearance was still changed. It might be possible that the acute feeling of sickness had then disappeared.

## PRACTICAL IMPLICATIONS

Resting and rumination may be used as promising behavioural indicators to be tested further for automatic disease detection in large dairy units. More detailed scientific work is needed, to explain the multiple changes in behaviours in different infections.

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## MILKING PROCEDURES HAVING AN IMPACT ON MILK SCC

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### SUMMARY

Herds with high somatic cell count (SCC) to adopt a short-term goal of reducing SCC as quickly as possible so that milk can be legally marketable and dairy cattle breeding sustainable. Cow's sire, enterprise, lactation, milking equipment and milking operator were fixed in data- base. The duration of each element of the working process was recorded. From these data analysis we can draw that adequate pre- milking preparation of the cow was essential to milk somatic cell count as well as over-milking ( $P<0.001$ ). The delay in application of the milking unit increased milk SCC ( $P<0.001$ ).

**Key words:** somatic cell count, milk quality, affecting factors, milking procedures

### INTRODUCTION

Livestock farming systems researchers have developed concepts, methods and tools to address the livestock farming activity as a whole. The success of the modern dairy farm will be dependent on the profitable production of high quality milk. Important factors for the prevention of high milk somatic cell count (SCC) are: post-milking teat disinfection, dry cow therapy, good milking management, treatment of clinical mastitis with antibiotics, and culling of problem cows (Barkema et al., 1998). Researchers (Merril et al., 1987) have determined that a twelve hours interval is the optimal milking interval in the case of twice-daily milking. A correct milking routine includes different working operations: cleaning udders and teats, manual pre-stimulation, fore-milking, attachment of milking unit to cow, removal of milking unit and effective post-milking teat disinfection (teat dipping). The investigations (Calhoun, 1995) have indicated that more than 50% of the working time is spent on milking. Improper and careless milking may result in decreased milk let-down, increased incidence of udder diseases and low milk quality, which are ultimately causing considerable economic loss for dairy producers.

SCC or a parameter derived from this, count is often used to distinguish between infected and uninfected quarters. It has been found that 200,000 cells/ml is the most practical threshold to determine the profitability of dairy farms (Heald et al., 2000; Rogers, 1995). Somatic cells are also present in milk of healthy cows, and the increase in SCC is a normal cellular defence against udder infections (Koivula, 2005). The year 1979 was the beginning of SCC registering in Estonia and from 1987 these data are measured and registered monthly in Estonian milk recording scheme (Pentjärv and Uba, 2004). More and more attention is paid to milk quality. After the accession of the Estonia to the European Union (EU), the demands on milk quality were in compliance with the EU legal limit of 400,000 cells/ml. Similar levels are required in New Zealand and Australia, where Canada established the requirement of a  $SCC<500,000$  cells/ml (Sargeant et al., 1998; Norman et al., 2000).

Estonia faces several problems with SCC affecting milk quality and udder health. In 2005, 26% of cows were culled due to udder diseases and mean milk SCC was between 361,000–435,000 cells/ml on dairy farms in Estonia (Jõudluskontrolli..., 2006). These circumstances give reasons to investigate the influence of milking procedures on SCC in milk.

## MATERIAL AND METHODS

Data were collected from five dairy cattle farms, where cows were milked with pipeline milking system. These agricultural enterprises were interested in monitoring and analysis of milkers' working time consumption, to make sure they follow the machine milking regulations. On three farms the cows were milked using the De Laval and on two farms with Rezekne pipeline milking equipment. The De Laval milking system was supplied with automatic cluster remover (ACR). On all dairy cattle farms the cows were milked twice daily.

Monitoring of the work activities of 24 milking operators, who milked the cows selected for our trials, was carried out immediately after control-milking. The duration of each element of the working process was recorded. Data on milk yield, fat and protein content and SCC were collected. The milking routine included the following:

1. Premilking udder and teats preparation (cleaning, washing, drying, massaging and stripping for early detection of clinical mastitis),
2. Cluster attachment,
3. Control of the milking unit and the cow's udder during milking,
4. Over-milking.,
5. Machine stripping,
6. Shutting off the vacuum and cluster removal from the cow,
7. Washing of the milking unit,
8. Washing hands and cloth towel,
9. Teat dipping in the sanitising solution immediately after milking.

Additionally were registered the "transition", i. e. the time a milking operator proceeded from one work operation to another, and the moments when the operator was involved in other activities. Stoppages and undone work operations were also fixed.

MS Excel and SAS program was used for data processing.

## EXPERIMENTAL DATA AND RESULTS

Present-day milking machines enable the operators to milk cows fast and so that all four udder quarters are milked out. At the same time the regulations of machine milking procedures must be followed. To ensure complete udder evacuation, it is essential to perform careful pre-milking udder preparation, which will induce the release of oxytocin (Bruckmaier, 1998). Randy et al. (1990) investigated milk SCC in 30 Virginia dairy cattle herds and were registered the udder preparation for milking. The analysis of the results revealed, that using of disposable paper towels reduces milk SCC, eliminating the possibility of bacteria transmission from infected cows to healthy ones. It is essential to attach milking unit to a cow promptly. When there is a durable delay in attach of milking unit then shorter will stay a milking time with milk ejection (Merrill et al., 1987; Barkema et al. 1998). After the unit attachment delay the milk ejection is slow, the udder is not emptied and the machine stripping is abnormally long-lasting (Etgen et al., 1987). If the cow is milked at the beginning and in the middle of lactation then it is suggested that the milking unit be attached within 50 seconds after beginning udder preparation. At the end of lactation, it is recommendable to attach the milking unit to a cow a little later (Bruckmaier, 2000).

**Table 1.** Durations of the basic milking procedures (sec)

Item8	Mean	Standard deviation	Minimum	Maximum
Udder preparation	24,0	6,8	11,0	49,0
Delay in milking unit attachment	5,9	8,9	0	47,0
Machine stripping	22,1	9,7	0	54,0
Over-milking	26,3	24,3	0	108,0

As soon as the milk flow has finished, the milking unit must be removed to prevent over-milking, which is one of the most considerable factor causing mastitis infection. Correct time for machine stripping is when the milk flow is changed incomplete and will start to decrease (Etgen et al., 1987; Randy et al., 1990; Timmermans, 1996). Over-milking may occur immediately after the unit attachment, when the cow's milk let-down reflex is not sufficiently initiated (Calhoun, 1995). Numerous studies have been carried out to investigate the optimum duration of machine stripping. Reneau (1986) recommends that the machine stripping last no more than 30 seconds, whereas Etgen et al. (1987), Barkema et al., (1998) and Calhoun (1995) suggest spending no more than 20 seconds. When the udder is prior adequately prepared for milking, 10–15 seconds of machine stripping per cow is sufficient.

If the udder preparation is insufficient, the machine stripping lengthens significantly (Etgen et al., 1987). Calhoun (1995) and Timmermans (1996) considered the long-lasting machine stripping to be characteristic to the work of these milking operators who devoted less time to udder preparation.

Over-milking of cows occurred with one milking operator using too many milking units i.e. four or more units in pipeline milking system (Calhoun, 1995).

Transition from conventional to machine milking increased the infection rate of mastitis. A teat dip was taken into use teat dip in the United States in 1916 to reduce risk of mastitis and the risk of spreading pathogens. Randy (1990), Nickerson et al. (1990), Etgen et al. (1987), Erskine et al. (1998) and Barkema et al. (1998) recommended to do post-milking teat dip to prevent udder diseases.

Schukken et al. (1992) were investigated the importance of post-milking teat dip in mastitis sickness reducing. In United States 74% of dairy cattle farmers applied post-milking teat dip. They considered essential to apply post-milking teat dip and designated that better results were got when pathogens were *Streptococcus agalactiae* and *Staphylococcus aureus*. Some authors as Randy et al. (1990), Timmermans (1996) and Erskine et al. (1998) recommended to apply post-milking teat dip in cows with increased SCC in milk. On the other hand, Roest (1995) considered useful that post-milking teat dip are applied in all milking cows in each milking immediately after cluster removing. Table 1 presents the duration of the basic work procedures of a milking operator. Operators were devoted to udder preparation meanly 24 seconds, which is less than physiological demands of machine milking. At that some cows were prepared only 11 seconds and the udder preparation was limited then reserved cleaning of teats and foremilk was not strip out.

Attachment of milking unit to a cow was often delayed.

Even 54 seconds were devoted to machine stripping. These milking operators, who economized in pre-milking udder preparation, devoted more time to machine stripping.

It was also studied how the milking operators are able to monitor milking units. As shown in Table 1, they did not manage following each milking unit with sufficient attention, as far as over-

milking was observed in several cows. The maximum duration of over-milking was 108 seconds. Teat dipping was used by 19 milking operators out of the 24 participating in the monitoring focused on the work operations of the milking operators. In all cases the method used for post-milking teat dipping was a teat dip in deso cup filled with the disinfectant solution.

Relationships between work operations performed during machine milking were studied (table 2).

**Table 2.** Connections between working operations doing in machine milking

Item	Machine stripping	Over-milking	Delay
Udder preparation	-0,294***	-0,429***	-0,235***
Delay in application of a milking unit	0,356***	0,432***	
Over-milking	0,597***		

\*\*\*P<0.001

The shorter was the pre-milking udder preparation, the more time took the machine stripping ( $r=-0.294^{***}$ ).

A significant positive correlation ( $r=0,356^{***}$ ) was observed between the delay in application of a milking unit and machine stripping. It is also apparent from the data in Table 2 that if milking operator ignores a single milking procedure, this will influence the rest of milking procedures as well.

**Table 3.** Correlation between the items

Item	Udder preparation	Delay in application of a milking unit	Machine stripping	Over-milking
SCC, $10^3/\text{ml}$	-0,304***	0,192***	0,267***	0,422***

\*\*\* – P<0.001

Correlation analysis was used to estimate the importance of a certain routine to be followed in a milking process. Table 3 reveals the correlations between the items. All the basic milking procedures influenced the milk SCC. A significant connection was between over-milking time and SCC ( $P<0.001$ ). Hence, the less the milking machines were followed, the higher was SCC per millilitre of milk, resulting from over-milking of teats. In practice, it is important to make sure how many milking units can be adequately handled by one milking operator during milking process.

A significant relationship was observed between udder preparation for milking and milk SCC. The shorter was the udder preparation for milking, the higher was milk SCC ( $P<0.001$ ). From these data analysis observed, that the delay in applying the milking unit influence the milk SCC ( $P<0.001$ ).

There was detected connection between machine stripping and milk SCC. Whatever more time a milking operator was spent to machine stripping then higher was milk somatic cell count ( $P<0.001$ ).



## CONCLUSIONS

The data analysis indicated that all the basic milking procedures affected milk SCC. A significant relationship was observed between over-milking and milk SCC ( $P < 0.001$ ). The shorter was the udder preparation for milking, the higher was the milk SCC ( $P < 0.001$ ), which proves that the delay in applying the milking unit to the cow has an impact on milk SCC ( $P < 0.001$ ). The more time a milking operator spent on machine stripping, the higher was the milk somatic cell count ( $P < 0.001$ ).

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## PHYSIOLOGICAL STATUS OF LIVE-STOCK UNDER THE INFLUENCE OF ABIOTIC ENVIRONMENT FACTORS

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**Key words:** *cattle, keeping, feeding, blood, vitamins, microelements, radionuclides*

### INTRODUCTION

During last fifteen years, the ecological state of the environment became worse, especially on a large area of the western region Ukraine. Based on the facts of the scientists, the quantity of toxic substances, which come into environment, was exceeded four hundred thousand names. The considerable widening of network of industrial enterprises, the increase quantity of dumps and shaft storehouse, using mineral fertilizer, herbicides and other chemical pesticides in excessive quantities. After the accident at Chernobyl AES, wide territories of Ternopil, Rivno and Volyn regions had suffered the pollution from 1 to 15 ki/km<sup>2</sup> by cesium – 137. Only in Volyn region, 167 populated areas had suffered such pollution, where 144188 of adult population and 46014 children were lived.

During last ten years, live-stock branch, especially cattle-breeding had suffered the decline. There are quite a number of reasons for that. They are – crossing period, change of property form, break down of collective economies, the unsuccessful attempt to change dairy live-stock into meat live-stock at polluted territories, mass rejection of live-stock, especially in many economies of Woodlands. Black-Polish stock was usually bred in this region, but now this breed is the most popular during last 10–20 years, which is adapted to the local conditions very good, and therefore it merits for saving [1, 2, 3, and 4].

But the indices of the productivity of this breed are very low in many economies as in Woodlands as in Lviv region.

Comparing three economies, the lowest milk productivity (during four years) was in cows from CAE “Galuzia” – 965 kg, a little higher from CAE “Svitanok” and the highest from JSC with Ltd. “Zvenyhorod” – 1740 kg. The birth-rate of calves from one hundred cows and heifers was low, and the most of newborn calves were hypotrophies.

And just these facts indicate that cows from these economies are not quite well. The reasons of low indices of productive and reproductive characteristics of these animals we should search for among the factors of the environment, because some of them have injurious action.

During the experiments, directly at live-stock farms, it is impossible to select the action of one factor on the organism. As a rule, they have complex influence on the organism, moreover, the action of one negative factor is frequently intensified by the other. And, as a rule, it takes place because of the deficiency of technological order, among their number is hypodynamia, non-balanced rations for lack of forage and their low quality (Zubets M.V., 2000), unilateral nourishment with a sort of fodder and others. The action of radioactive irradiation, such as toxic chemicals, deficiencies in feeding and keeping, have a harmful influence on the blood-forming organs, immune prevention and animals ability to reproduce.

## MATERIALS AND METHODS

Taking into account all high-mentioned thoughts, the purpose of our investigations dealt with the studying of the interior peculiarities of the live-stock organism of Black-Spotted breed by the influence of combined action of unfavorable (ecological, climatic and technological) factors of the environment on exterior indices, morphological and biological content of blood and their changes depending on seasonal and climatic conditions.

It was determined that based on unfavorable factors of the environment, which had a regular influence on animals organism in the economies where the investigations were done, were approached to a certain extent but had different strength. For the most economies the most powerful source of environment pollution was and is still regular, in small doses, ionized reaction: on the territory of CAE “Prominj”, “Majak” and “Nadsluchanska” of Rivne region and “Zbruch” and “Majak” of Ternopil region from 1 to 15 Ki/km<sup>2</sup>, “Galuzia” – from 1 to 5 Ki/km<sup>2</sup>, “Svitanok” and Volynj pedigree association – to 1 Ki/km<sup>2</sup>. The territory of the rest economies refers to a conditionally clean zone.

Hygienic conditions of animals, including the main parameters of microclimate in rooms were satisfactory. In summer, cows were pastured in the pasture-grounds, in winter they were kept on a leash, and bulls were kept in boxes without leash during a year. Animals providing with fodder didn't correspond to hygienic and feeding norms at the most farms. Ration supplying with fodder units for cows in some economies was only 63 per cent concerning needs, digestible protein – 54 per cent, sugar – 34 per cent, phosphorus – %:%, carotene – 32 per cent and some microelements from 25 to 50 per cent. Systematic moving from machine milking to hand, non-rhythmical serving of feed to farm and other violation of technological order were constantly active stressors except long-term hypo dynamics.

Therefore, the conditions of animals keeping and feeding at farms didn't correspond to the physiological needs of organism.

## RESULTS AND DISCUSSION

All unfavorable factors of the environment which were active during long period of time and were the cause that after the hibernation, no economies had cows, which living mass didn't correspond to the standard demands for Black-Spotted breed. Cows from JSC with Ltd.”Zvenyhorod” had the biggest living mass – 431,8 kg and the least – 347,3 kg from CAE “Galuzia”. All cows were undersized. Comparatively to the standard, all animals had higher index of long-leg, especially in cows from zone which is polluted with radionuclide. All animals had dramatic reduced overall size.

This information testifies dramatic decrease of meat quality of cows. But combined action of unfavorable factors of the environment had considerably higher influence over animals from the economies which were in polluted zone, and considerably lower in Lviv region.

Studying the influence of season on the indices of cows' blood, it was determined, that seasonal wavering is inherent for the animals from the most economies. In spring, after the end of stall period the quantity of leucocytes, common protein, alpha-globulins, the concentration of haemoglobines, the level of ceruloplasmine, and also cholesterine and carotene was decreased in blood.

Therefore, spring period directly before the beginning of pasture keeping is a period, when all deficiencies of winter stall keeping are shown. Animal's organism is weak because of the absence

of active motions and inferior feeding during this period of time. During the period of pasture keeping, a number of physiological and biochemical indices of blood partly increase to the level of physiological norm. But for animals, which during winter-stall period were kept in the conditions of underfeeding, summer period with pasture keeping is insufficient for absolute restoration of the level of indices number. Therefore, animals come into hibernation with the violation of metabolism.

Thus, from all factors, which have a harmful influence on functional state of cows' organism, feeding factor was the most powerful, it means non-balanced feeding.

Now, it isn't studied enough about the influence of chronic action in little doses of radiative irradiation on live-stock organism. With this purpose, on the base of two economies "Majak" and "Prominj" in Rivne region, it was studied the indices of cows blood age of which was from 11 to 7 years, part of them was of the same age at the time of Chernobyl crash. The results show the violation in organism of all age cows group of metabolic processes, because of long-term action of unfavorable factors on organism during several years, non-balanced feeding was leading among them, especially in CAE "Prominj".

Though, the content of common milk was in the limits of physiological norm, but albumine – globuline correlation was violated. Abruptly reduced content of albumines and alpha-globuline fraction due to increase beta- and gamma-globulines. The content of immunoglobulins was high especially in those, which were kept in zone for nine years and the concentration of alkaline phosphatase was higher into 2, 5 times. Such indices of blood as the quantity of erythrocyte, the concentration of hemoglobin's and haematocritic value were low.

Similar regularity was also in the indices of blood in cows from CAE "Majak".

In spite of all these problems and difficulties, the agricultural activity is an integral part of inhabitants' life in radioactive polluted territories.

Many scientists, especially Kovalyshyn V.I. and co-authors, 1995; Koroljov A.A. and co-authors had proved, that the necessity of Vitamins and microelements, under such conditions, have to be increased, in comparison with physiological norm into 2–2,5 times.

Taking into account, that non-balanced feeding had weighty harmful factors and taking into consideration the information from literature, we decided to study the effect of trivitamine injection to animals and their foddering with poly-salts of microelements. It was determined, that after winter period of keeping, during which cows from the experimental group were injected with trivitamine and were fed with poly-salts of microelements, the concentration of common protein, albumin and globulin fraction, immunoglobulin, hemoglobin, ceruloplasmine, phosphorus of ATP, carotene and DFA reaction was increased in cows from CAE "Svitanok", and the quantity of erythrocyte, the concentration of non-organic phosphorus glucose and cholesterine was decreased.

In experimental groups, in cows from CAE "Galuzia" the level of common protein, alpha- and beta-globulin, hemoglobin, ceruloplasmine, phosphorus ....

It was decreased the level of carotene, hemoglobin and ceruloplasmine in cows from the control group.

The level of carotene was considerably raised in plasma of cows in all economies after summer period of pasture in "Svitanok" – 1011 mkg/1000 ml of control and 1076 mkg/1000ml in experimental group in "Galuzia" – 923 and 976 – D.

The results of other investigated indices had enough large oscillation amplitude in cows of as experimental as control group. But under the influence of trivitamine and poly-salts of microelements, it was defined the tendency to some normalization of the indices.

Using trivitamine and poly-salts of microelements favour insignificant rising of the concentration of globulin, erythrocytes in blood and decreasing of haemocritic value in bulls.

Leucocytes, higher concentration of common protein, albumins, alpha-beta and gamma-globulin and also ceruloplasmine were present in the blood of new-born calves from the cows which were got trivitamine and poly-salts of microelements. Calves from CAE "Svitanok" had higher ORE.

Therefore, the information showed that parenteral injection with trivitamine and animals feeding with poly-salts of microelements has positive action under the conditions of insufficient feeding, as during using as during far-off terms.

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## SELENIUM-DEPENDENT GROWTH INHIBITION OF MASTITIS PATHOGENS IN COW'S MILK

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### SUMMARY

Adequate selenium (Se) status is needed for maintenance udder health of dairy cows. However, the mechanisms by which Se is supporting health of cows' udder are still unclear. Current work is focused on Se-dependent growth suppression of mastitis pathogens in bovine milk.

**Keywords:** selenium, bovine, mastitis, antimicrobial effect

### INTRODUCTION

Bovine mastitis is a disease of major welfare concern, causing high economic losses today's dairy industry. Use of antimicrobial drugs has not solved mastitis which appears to be as common today as before the introduction of antimicrobial drugs. Knowledge about factors and mechanisms influencing immune defense of cows' udder is giving a possibility to improve udder health. Adequate Se status is one of the factors needed for maintenance udder health of dairy cows. However, the mechanism by which Se promotes better health of mammary gland is still unclear. Objective of our study is to clarify the role of Se in mammary gland defense. Study is focused on the Se-dependent growth suppression of mastitis pathogens in milk.

### MATERIAL AND METHODS

Two Se feeding experiments (studies 1 and 2) were carried out on Se-deficient dairy cows in Estonia. Cows were allocated into Se-supplementation (n=25 in study 1, n=39 in study 2) and nonsupplemented groups (n=25 in study 1, n=16 in study 2). The Se-supplementation groups received 0.2 ppm organic Se in the form of Se-yeast in their daily diet for 8 weeks. The nonsupplemented cows received their standard diet with no Se supplementation. Blood and quarter milk samples were collected before and after 8 weeks Se supplementation. From whole blood selenoenzyme glutathione peroxidase (GPx) activity was analysed. Microbiological examination and analyses of somatic cell count were carried out from milk samples. Whey was prepared from milk of 12 cows without signs of mastitis during 8 weeks experimental period for analyses of Se-dependent antimicrobial factor. Samples originated from study 2. Whey was fractionated by high-performance liquid chromatography in purpose to localize Se-dependent antimicrobial component. Fractions were collected between 1 minutes interval and tested for

growth inhibitory activity against *S. aureus* strain by using turbidimetric method (Malbe et al. 2006).

## RESULTS

Mean GPx activity of all cows was low before starting of Se-supplementation, being 0.232  $\mu\text{kat/L}$  Hb in study 1, and 0.645  $\mu\text{kat/L}$  Hb in study 2. Se-supplementation during 8 weeks increased mean GPx activity to 3.014  $\mu\text{kat/L}$  Hb in study 1, and to 3.867  $\mu\text{kat/L}$  Hb in study 2. GPx activity in control groups remained low. In study 1, percentage of udder quarters infected with mastitis pathogens decreased from 22.9 to 13.0 in Se-supplemented group and increased from 21.3 to 25.6 in nonsupplemented cows (Malbe et al. 1995). In study 2 Se supplementation was effective in helping to maintain quarters uninfected. Cows' udder was more prone to be infected when GPx activity in blood was below 3.3  $\mu\text{kat/L}$  Hb (Malbe et al. 2003). Also, whey became *S. aureus* growth inhibiting in cows whose GPx activity in blood exceeded 4  $\mu\text{kat/g}$  Hb. Inhibition of growth rate of *S. aureus* was traced in two fractions out of 8 studied in detail (Study 2, Malbe et al. 2006).

## DISCUSSION

Results of our studies indicate that daily supplementation of 0.2 ppm Se in the form of Se-yeast increase host defence against invading bacteria. However, the effect of Se-supplementation on mastitis pathogens depends on severity of Se-deficiency on cows. Study 1 was organized on cows with severe Se-deficiency and Se-supplementation had clear effect on improvement of pathogens infected udder quarters. On cows with moderate Se-deficiency in study 2, Se-supplementation was mostly effective in mastitis prophylaxis and had little or no effect on improvement of those udder quarters which were pathogen-infected at the start of the study. Eight weeks Se-supplementation induced changes in milk composition and whey became *S. aureus* growth restricting. The growth of *S. aureus* was inhibited by whey fractions from animals whose GPx activity in blood exceeded 4  $\mu\text{kat/g}$  Hb. This significant inhibiting effect on bacterial growth suggests that there is Se-dependent antibacterial activity in whey. Our findings are in accordance with earlier report that Se-supplementation had an inhibiting effect on *in vitro* bacterial growth in whole whey of cows (Ali-Vehmas et al. 1997).

## CONCLUSION

Adequate Se status in cows' is needed for suppressing growth of mastitis pathogens in milk, which is possibly connected with presence of Se-dependent antibacterial component in whey.

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## **CUBICLE DIMENSIONS AFFECT RESTING-RELATED BEHAVIOUR, INJURIES AND DIRTINESS OF LOOSE-HOUSED DAIRY COWS**

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### **SUMMARY**

Increasing cubicle dimensions reduced the estimates of prevalence of prolonged movement sequences and general dirtiness of cubicle loose-housed dairy cows in predictive regression models. Neck rail height, distance of neck rail from the curb and availability of head lunging space were the statistically significant predictors. Despite the relatively low R-squared values in the predictive regression models, the results of our pilot study encourage further research on the subject.

**Keywords:** cubicle housing, lying-down behaviour, standing-up behaviour, skin lesions, cleanliness, animal welfare

### **INTRODUCTION**

Loose-housed dairy cows are most often kept in cubicle (free stall) systems. A dairy cow spends 40–60% of time lying down (Tucker et al., 2005), and lies down and stands up 10–17 times each day (Wechsler et al., 2000). Consequently, cubicle properties, such as dimensions and surface material, have high potential to affect welfare of the cows through hindering normal behaviour (Veissier et al. 2005) and causing injuries (Livesey et al., 2002) or dirtying (Chaplin et al., 2000). In our pilot study we concentrated on examining the influence of different cubicle dimensions on the lying-down and standing-up duration, as well as some health variables in loose-housed dairy cows on Finnish commercial dairy farms.

### **MATERIAL AND METHODS**

The study was carried out on 27 commercial dairy farms located mainly in the North Savo region of Finland during the winter feeding period of 2005 (January–April). The farms housed 28–92 (mean 52 ± S.D. 17) milking cows in insulated cubicle barns. In total, there were 1192 cows in the herds (68% Finnish Ayrshire and 32% Finnish Holstein-Friesian). All farms had cubicles with mats, and used either a small amount of wood shavings (n = 22), peat (n = 2) or straw (n = 1) or nothing (n = 2) as litter.

Lying-down and standing-up behaviour of cows was observed for a total of three hours on each farm. Observations were made directly for 1.5 hours after morning milking and 1.5 hours before evening milking. During the observation periods, the duration (seconds) of all observed lying-down (n = 23–75 / farm) and standing-up (n = 21–75 / farm) sequences (individual cows could not be identified) were measured with a stopwatch. Timekeeping was started for a lying-

down sequence when a cow bent her foreleg and stopped when she settled down to a lying position. For standing-up sequence, the timekeeping began when a cow started to pull her feet under herself and made a forward swinging motion with her head, and stopped when she was standing in balance on all four feet. Winckler et al. (2003) have suggested that movement sequences lasting over seven seconds in cattle can be considered prolonged or abnormal. Using this criterion, prevalence of both prolonged lying-down and standing-up sequences in each herd was calculated. Observations of movement sequences longer than 100 seconds ( $n = 10$ ) were then excluded from the data in order to prevent the few extremely large values from skewing the average, and average duration (seconds) of movement sequences in each herd were calculated.

A random sample of cows ( $n = 10$  / farm) was pre-selected for clinical examination of cleanliness and injuries. Cleanliness of feet, udder (posterior view), underbelly and udder (lateral view) and thighs of each cow were scored as 1 = “clean”, 2 = “under 50% of the area soiled” or 3 = “over 50% of the area soiled” on both the left and right side of the cow, where applicable. The separate scores for each location were summed up to form an “overall” dirtiness score (theoretical minimum 7 = perfectly clean, and maximum 21 = extremely dirty) for a cow. Based on the individual cow scores a herd average dirtiness score was calculated. Injuries in the knees (carpal joint), hocks (tarsal joint) and neck were scored as 0 = “no hairless patches, scabs or wounds”, 1 = “hairless patches”, 2 = “scabs and/or wounds” or 3 = “swelling in the joint / area”. All joints were examined but only the cow’s highest score for a given location (knee or hock) was taken into account. Prevalence of severe injuries in hocks and knees on a farm was calculated as a percentage of cows observed to have scabs, wounds or swelling. For prevalence of neck injuries also cows with hairless patches were included in the calculation.

The behaviour, cleanliness and injury variables (Table 1.) were used as dependent variables in multiple linear regression analysis (SPSS for Windows 14.0). Cubicle dimensions (Table 2.) were always included as independent variables in the analyses. Table 2 lists also the additional independent variables used in the analyses of injury and dirtiness variables: the age and milk yield of the injury- and cleanliness-sampled cows (all variables), feeding barrier type (neck injuries) or pen floor type (dirtiness score). Backward regression selection was used with the least significant predictor at each step being removed from the model, until the remaining predictors were all significant. The significance threshold for removal from the model was  $P = 0.1$ .

**Table 1.** Descriptive statistics of the behaviour, injury and dirtiness variables used as dependent variables in the regression analyses. Figures are averages of the 27 farms.

Variable	Mean $\pm$ S.D.	Min	Max
Average lying-down duration (sec)	6.1 $\pm$ 0.7	4.6	7.6
Prevalence of prolonged lying-down sequences (%)	17 $\pm$ 7.9	4	35
Average standing-up duration (sec)	7.0 $\pm$ 1.8	4.6	11.6
Prevalence of prolonged standing-up sequences (%)	24 $\pm$ 14	3	57
Prevalence of severe hock injuries (%)	51 $\pm$ 20	0	80
Prevalence of severe knee injuries (%)	42 $\pm$ 22	0	80
Prevalence of neck injuries (%)	30 $\pm$ 30	0	80
Dirtiness score <sup>1)</sup>	10.2 $\pm$ 0.9	8.8	12.8

<sup>1)</sup> High score denotes dirtier animals.

**Table 2.** Descriptive statistics of the cubicle dimensions and other variables (cow age and yield, feeding barrier and floor type) used as independent variables in the regression analyses. Figures are averages of the 27 farms.

Variable	Mean $\pm$ S.D.	Min	Max
Cubicle width (cm) (CW)	112.8 $\pm$ 2.1	108	118
Cubicle length (cm) (CL)	225.8 $\pm$ 10.0	205	250
Neck rail height (cm) (NRH)	106.1 $\pm$ 7.2	85	114
Distance of neck rail from the curb (cm) (NRD)	159.0 $\pm$ 13.1	118	190
Median age of cows (years) (AGE) <sup>1)2)3)</sup>	4.2 $\pm$ 0.7	2.8	5.8
Median of yearly yield of cows in 2004 (kg milk) (YIELD) <sup>1)2)3)</sup>	8031 $\pm$ 1239	5044	11041
	<b>Variable coding in regression analysis</b>		
	<b>1</b>	<b>2</b>	
Availability of head lunging space in front of the cubicle (LS)	No, n = 21	Yes, n = 6	
Feeding barrier type (FB) <sup>2)</sup>	Gates, n = 18	Neck rail, n = 9	
Floor type (FLOOR) <sup>3)</sup>	Slatted, no rubber, n = 17	Solid, no rubber, n = 10	

<sup>1)</sup> Median age and yield of the injury- and cleanliness-sampled cows (n = 10) on each farm. <sup>2)</sup> Variable used in the analysis of injury. <sup>3)</sup> Variable used in the analysis of dirtiness.

## RESULTS

Predictive regression models for examined behaviour, injury and dirtiness variables are presented in Table 3. According to the model, increasing neck rail distance from the curb decreased the prediction for the average duration of lying-down sequence, as well as prevalence of prolonged lying-down sequences. Average duration of standing-up sequences was not accounted for by any of the cubicle dimension variables. Prediction for the prevalence of prolonged standing-up sequences decreased by increasing neck rail height and distance of neck rail from the curb, and by providing the animals with lunging space in front of the cubicle.

Prediction for the prevalence of severe hock injuries was increased by lunging space in front of the cubicle and the age of the cows. Prevalence of severe knee injuries could not be predicted by any of the cubicle dimension variables, but it increased with increasing milk yield. Prevalence of injuries in the neck was predicted by the height of the neck rail and the type of feeding barrier ( $R^2 = 81\%$ ), but these two variables had a significant mutual correlation ( $r_s = -0.43$ ,  $P < 0.01$ ), and could not be used in a common model. Separate regression analyses with these two independent variables revealed that feeding barrier type was a better predictor for neck injuries ( $R^2 = 79\%$ ,  $P < 0.001$ ) than neck rail height ( $R^2 = 27\%$ ,  $P = 0.005$ ). Therefore, we compared the difference in neck injury prevalence only between the feeding barrier types. Farms with head gates (n = 18) had significantly ( $P < 0.001$ , Mann-Whitney test) lower prevalence of neck injuries ( $11 \pm 13\%$ , mean  $\pm$  S.D.) than farms with neck rail (n = 9,  $66 \pm 16\%$ ). Increase in the cubicle width and the age of the cows lowered and solid floor increased the prediction for the herd average dirtiness score.

Regression models accounted for between 0 and 44% of variability (R-squared values) in the investigated variables (Table 3.).

**Table 3.** Predictive models obtained from multiple linear regression analyses for behaviour, injury and dirtiness variables used in the study.

Variable	Model <sup>1)</sup>	S.E. <sup>2)</sup>	R <sup>2</sup> (%)
Lying-down duration	12.5-0.04×NRD***	0.7	40
Prevalence of prolonged lying-down sequences	68.7-0.33×NRD**	6.77	29
Standing-up duration	7.39	2.0	0
Prevalence of prolonged standing-up sequences	202-11.4×LS <sup>o</sup> - 0.73×NRH* - 0.55×NRD*	12.5	32
Prevalence of severe hock injuries	-15.7 + 26.1×LS** + 8.24×AGE <sup>o</sup>	16.8	35
Prevalence of severe knee injuries	-15.5 + 0.01×YIELD*	20.0	17
Dirtiness score	25.2-0.12×CW <sup>o</sup> -0.58×AGE** + 0.83×FLOOR**	0.72	44

<sup>1)</sup> Model including the constant. Significance of a variable in the model: <sup>o</sup> P < 0.1, \* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001. See Table 2 for abbreviation keys. <sup>2)</sup> Standard error of the model estimate.

## DISCUSSION

In our study, the placement and height of the neck rail were the most important cubicle dimensions predicting the lying-down and standing-up behaviour of dairy cows. Tucker et al. (2005) did not find clear preference for cubicles with less restrictive placement of neck rails in non-lactating Holstein cows. Nor did the neck rail placement affect the cows' lying time in the cubicles. When the neck rail was placed low or close to the curb, however, cows spent less time standing with all four feet in the cubicles (Tucker et al., 2005). Thus it is likely that the neck rail placement affects unfavourably mainly behaviours that involve having to stand in the cubicles: i.e. lying-down and standing-up behaviour in the current study.

Interestingly, both average duration of lying-down sequence and prevalence of prolonged movement sequences were predicted by the distance of neck rail from the curb, but duration of standing-up could not be predicted by any of the variables used in the models. To our knowledge, the effect cubicle dimensions on the duration of lying-down and standing-up movements has not been subject to research. Especially the prolonged movement sequences would merit further investigation as possible welfare indicators in cubicle housed cattle.

Providing lunging space in front of the cubicle and the rising age of the cows increased prediction for prevalence of severe hock injuries. The size of a cow usually increases with age, and large cows have more severe hock injuries than smaller cows (Haskell et al., 2006). However, proper lunging space is associated with ease of movement (McFarland, 2003) and reduced rate of injury (Anderson, 2003, Haskell et al. 2006) in dairy cows. Therefore, the influence of lunging space on the prediction of hock injuries in the current study is confusing, but it may be due to some variable that was not accounted for in our study. In addition, high milk yield increased the prediction for prevalence of severe knee injuries. In contrast with our result, Haskell et al. (2006) found that low level of milk production was associated with more knee swellings in cows in cubicle housing on commercial dairy farms. It cannot be determined if this could be due to differences in e.g. farm management between the two studies.

Farms with neck rails at the feed bunk had a higher prevalence of neck injuries than farms using head gates. Therefore, the placement and design of the feeding barrier is important, and should be taken into consideration while designing cow facilities to prevent undue injuries to the animals. The height of neck rail in cubicles could not be used as a predictor in multiple regression

analysis due to multicollinearity with the feed barrier type (farms with neck rails at the feed bunk had lower neck rails in cubicles), but it is likely that also the height of the neck rail in cubicles can affect the formation neck injuries in dairy cows.

Wider cubicles are more likely to become soiled with manure than narrow cubicles (Tucker et al., 2004), and thus influence animal cleanliness. However, in our study, cubicle width had a tendency to lower the prediction of the overall dirtiness score of a farm. Cow age was also a lowering factor. The reason for these effects is not clear, but they could be caused by differences in the farm management. Solid floor in the barn alleys increased the estimate of the dirtiness score compared to slatted floor. Slurry accumulates more easily on solid than slatted floors, affecting cleanliness of the cows' legs. Indeed, in our study the solid floor farms had a slightly worse leg cleanliness (data not shown).

Cubicle length was not a predictive variable in any of the models. Neither did cubicle width turn out a significant predictor. The variation in these cubicle dimensions on the farms might have been too low to bring out any significant effects.

Overall, increasing cubicle dimensions reduced the dirtiness score and the prevalence of prolonged movement sequences in the predictive models. The neck rail height, the distance of the neck rail from the curb and the availability of head lunging space were the statistically significant predictors. The regression models explained a rather low proportion of the variability in the data, and produced some unexpected results, which means further research with additional explanatory variables is needed. Cubicle surface material has an important effect on the investigated behaviour, injury and dirtiness variables, as has been shown in previous studies (e.g. Herlin, 1997, Chaplin et al., 2000, Wechsler et al., 2000, Livesey et al., 2002, Haskell et al., 2006). Although the effect of cubicle surface material was excluded to some extent from our study by having farms with mats only, differences in the surface management (e.g. regularity of cleaning and adding new bedding) may still contribute to the formation of lesions or dirtiness (Veissier et al., 2004).

In conclusion, our results indicate that cubicle dimensions affect especially resting-related behaviour, but also injuries and dirtiness of loose-housed dairy cows. Despite the relatively low R-squared values in the predictive models, the results of our pilot study encourage further research.

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## HEAVY METALS POLLUTION (PB, CD) AND ITS INFLUENCE ON ANIMALS RAISED IN A NON-FERROUS ORE PROCESSING UNIT

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### SUMMARY

In an area of a non-ferrous ore processing unit it was taken in view the lead and cadmium concentrations.

The metals were harvested from forages, organs and bone samples from animals of different species and ages (cattle, horse and goat youth and adults).

Test was made by atomic absorption spectrophotometer and results interpretation was in compliance with the provisions of the stipulated standards.

The results revealed exceeding of admitted limits in all samples (from forages, organs or bone).

The high lead and cadmium concentrations in organs and bones, correlated by clinic and forensic examinations show a chronic poisoning in this area.

**Keywords:** pollution, lead, cadmium, organs, forage, limits, samples

### INTRODUCTION

The organizing and developing a monitoring system of the whole environment and its component represents the beginning of the national protecting activity of environment as an efficient and clear state politics.

Pollution represents the human beings actions of staining his own life area, clearing this environment being a natural law which permits the continuous activity of life.

Chemical pollution is a consequence of industry development in some areas with implication on health status of organisms and environment.

Such a zone is one surrounding the site of a non-ferrous ore processing unit, in the centre of the country.

This unit pollutes the area with a series of chemical elements and lead and cadmium, too.

Lead spreads the animal organism by the digestive way (forage, waters) or respiratory way; it is fast absorbed, blocks the access of  $\text{Ca}^{2+}$  and modifies the chemical processes which rely on it. It blocks the porphyrinic chain, changes the red blood cell membrane and favors the haemolysis. Lead has a toxic action on the central nervous system, vessels and different organs.

Cadmium, an other pollutant element studied in the present paper, enters the organism by the digestive way; a part of it passes the vessels being carried by the RBCs and deposits especially in liver, kidney and spleen.



Cadmium stops the oxidative phosphorylation and favors the iron excretion, producing anemia.

### MATERIALS AND METHODS

The researches had in view the establishing of heavy metal concentration (Pb, Cd) in forage (hay, maize, lucerne), organ (liver, kidney, spleen) and long bones samples, from animal raised in a non-ferrous processing unit area.

The organ samples were taken from animals of different species and age category (young cattle and goats, adult cattle and horses).

The analysis of lead and cadmium concentration was made by atomic absorption spectrophotometry.

The results interpretation was made by 97/2005 Order for organ and bone samples and 120/2005 Order for forages.

### RESULTS AND DISCUSSION

In table no. 1 there are shown the average values of lead and cadmium concentration in forage samples (hay, maize, lucerne).

**Table 1.** The average Pb and Cd concentration in forage samples

Kind of sample	No. of sample	Determined element	
		Pb	Cd
Hay	10	119.6	4.15
Maize	20	0.92	0.16
Lucerne	20	161.6	6.25
Maximum admitted limits 120/2005 Order	Hay, lucerne	10	1
	Maize		

Analyzing the data in the table, it could notice an exceeded concentration of Pb by 11.96 times beside the stipulated 120/2005 Order in hay samples and by 16.16 times in lucerne samples.

Regarding cadmium concentration in forages, it was noticed a four times higher value in hay and 6.25 times in lucerne.

In maize samples, Cd and Pb concentrations are framed within the admitted limits.

The pollutants enter the plants in stomata, producing the poisoning of the chloroplasts, ribosomes and other cell compounds, reducing the main processes of life. So, these plants, eaten by animals could produce poisoning. The average lead concentrations in organ and bone samples are shown in table 2.

**Table 2.** The average Pb concentrations in bone and organ samples harvested from animals in a non-ferrous ore processing unit limitrophe area

Species	Type of sample				
	liver	kidney	spleen	heart	long bone
Young goats	0.77	0.24	0.59	–	5.69
Horses	18.90	18.94	25.2	11.84	101.2
Adult cattle	8.18	11.86	10.6	6.7	120.2
Calves	16.16	6.15	0.504	–	33.79
Maximum admitted limits 97/2005 Order	0.5	0.5	0.5	0.5	3.0

Following the data in the table, it could be noticed an exceeding of the admitted limits in most of the samples, no matter the species, age or organ.

Thus, in young goats, the exceeding was higher by 1.54 times in liver, 1.01 in spleen and 1.89 times in bones. There was not recorded any exceeding in kidney. In adult horse organ samples, the exceeding was 37.8 times higher in liver and kidney, 50.4 times in spleen, 23.86 times in heart and 33.73 times in bone samples.

In adult cattle samples, the exceeding was 16.36 times higher in liver, 23.78 times in kidney, 21.2 times in spleen, 13.4 times in heart and 40 times in bone.

In young cattle samples, lead concentration exceeded the maximum admitted limit by 32.32 times in liver samples, 12.3 times in kidney and 11.26 times in bone.

On the basis of obtained results it could be noticed that lead is placed into liver, kidney, spleen and after a while it accumulates in bones.

At a nephral level, lead inhibits the activity of some mitochondrial hydrogenases and destroys the pyruvic acid.

Average cadmium concentrations in bone and organ samples are shown in table 3.

**Table 3.** The average concentrations of cadmium in organ and bone samples harvested from animals in a non-ferrous ore processing unit limitrophe area

Species	Type of sample				
	liver	kidney	spleen	heart	long bone
Young goats	0.023	0.004	0.021	–	0.011
Horses	18.49	33.88	8.73	2.47	2.18
Adult cattle	3.19	7.95	0.23	0.17	0.09
Calves	0.45	0.782	0.213	–	0.10
Maximum admitted limits 97/2005 Order	0.5	1.0	0.5	0.5	1.0

Regarding the data in the table, the concentration of cadmium recorded an exceeding beside the admitted limit stipulated by 97/2005 Order, in organ and bone samples of adult cattle and horses.

Thus, in liver samples, the exceeding was 37 times higher in horses and 6.38 times in cattle; in kidney it was 34 times higher in horses and 8 times in cattle.

In spleen and heart samples it was recorded an exceeding only in horse samples, respective 17.46 times higher in spleen and 4.94 times in heart. In bone samples it was 21.8 times higher.

The excessive cadmium increases the lead toxicity and it has negative effects on reproductive system, skeleton, neural system and haematopoiesis.

There is a large cadmium and lead pollution in the non-ferrous ore processing unit area. These pollutants exist in forages and animal organisms, no matter the species and age. The highest

concentrations were carried out in the samples from adult animals and especially horses, cattle being more resistant to cadmium pollution.

Clinical and forensic examinations, correlated with the high concentration of lead and cadmium in organs and bones show a chronic poisoning with the two heavy metals in the studied area.

### CONCLUSIONS

1. Lead concentration in forage samples exceeded the admitted limit by 12–16 times and cadmium concentration by 4–6 times.
2. Lead recorded an exceeding beside the maximum admitted limits in organ and bone samples of young goats by 1–2 times, of adult horses by 24–50 times, of adult cattle by 13–40 times and of young cattle by 11–32 times.
3. Cadmium recorded values exceeded the maximum admitted limit in all horse samples by 5–37 times, in a decreasing order in: liver, kidney, bone, spleen, heart.
4. In cattle, cadmium concentration recorded an exceeding beside the admitted limit by 6–8 times in liver and kidney.
5. A large pollution with lead and cadmium is noticed in the limitrophe area of the non-ferrous ore processing unit.
6. The high concentration of lead and cadmium in organ and bone samples correlated with the clinical and forensic findings show a chronic poisoning with the two heavy metals in this area.

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## PREVENTION OF WOLF AND OTHER LARGE CARNIVORE DAMAGES IN FINNISH LIVESTOCK HERDS USING LIVESTOCK GUARDING DOGS (LGD)

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### SUMMARY

Phase 1 of studying a cost-effective method of protecting livestock with LGD for Northern Europe and the Baltic Sea Area included contacting early LGD adopters, semi-structured interviews and in-site-visits to Finnish farms in summer 2006. 4 farms located in traditional large carnivore areas in eastern and 4 in central parts of Finland. Total numbers of dogs were 31 including 19 LGDs (1–4 per farm) of 8 different breeds. The experiences were encouraging: no predation since LGD(s) presence, only minor difficulties. Herding dogs and LGDs got along acceptably. LGDs had also multifunctional influence e.g. increasing the general feeling of security.

**Keywords:** livestock, livestock guarding dogs, carnivore damages, wolves

### INTRODUCTION

Successful pasturing is essential in cattle breeding both from an economic and cattle welfare point-of-view. (e.g. European Convention for the protection of animals kept for farming purposes, CETS No.87). For organic farming the principles of natural and sustainable measures are important due to reputation factors as well as regulation factors (Council Regulation (EEC) No 2092/91).

In the latest decades the populations of wolf, bear and other large carnivores have increased and expanded towards more inhabited areas in all of Europe (Management Plan for the Wolf Population in Finland, MMM 2005). The amounts of damage of pasturing livestock and hunting dogs are increasing. The management of wolves and other large carnivores (except the reindeer herding area in northern Finland) is regulated by the EU nature directive attachment IV. Measures for prevention of wolf and other large carnivore damages have been supported from state finances. The methods used in Finland so far have included fencing of pastures, dog yards and wolf phone service. Farms have started spontaneously to acquire LGDs. At the same time the pedigree dog registration statistics of the Finnish Kennel Club also shows increasing numbers of LGDs. However there is no exact knowledge of the numbers of LGDs already used for livestock protection in Finland. Thus, it is important to explore the suitability and cost effectiveness of this method of damage prevention.

LGDs have been used for centuries. They work by staying with the livestock and driving away intruders, any need for physical conflict rarely occurs (Rigg 2004). Often there is a need for more than one dog to keep up the necessary level of protection. In United States, LGDs have been introduced as a new method of guarding flocks. Research was initiated during the late 1970's by

several organizations to evaluate the use of guarding dogs to protect livestock from coyotes (*Canis latrans*) and dogs. In general LGDs were capable of reducing predation on sheep in a variety of management systems. (Linhart et al. 1979; Mc Grew and Blakesley 1982; Coppinger et al. 1983; Green and Woodruff 1983a, 1983b; Black and Green 1985).

There is no LGD tradition in Finland, partly due to the fact that there has not been any need for extensive protection against large carnivores during the last decades. As the amount of wolf damages towards flocks and hunting dogs increase, exploring possibilities of using LGDs becomes even more important (Dawydiak & Sims 2004).

In the Nordic countries, the use of LGDs for protecting sheep has been tested in Norway (Hansen and Smith 1999). However, Norwegian methods are not directly applicable to Finnish conditions as Norwegian sheep are widely dispersed on an open range. In Sweden, the testing of LGDs in electric fenced areas has started recently (Levin 2005).

LGDs should be kept with, brought up with, socialised with and bonded with the stock it is going to protect (Coppinger 1992). As the pasturing season lasts only half the year in Finland, it is especially important to find the correct balance between the dog's level of bonding to the family and to the livestock. Moreover, LGDs need proper basic obedience training and the dog needs to be socialized to people and to places outside its own territory (Davydiak & Sims 2004, Koljonen 2002). Also it should be paid attention to so called everyman's right i.e. the public right of access to forests and other land enacted by Finnish law.

The price for a LGD pup bred in Finland is approximately 1000 € and for an imported one approximately 1500 €. Taking into consideration all costs for food, vaccinations, maintenance and for possible insurance and healthcare yearly costs are approximately 500–1000 €, depending on the health status of the dog (Management Plan for the Wolf Population in Finland, MMM 2005).

Purchasing Livestock Guarding Dogs to protect livestock in wolf areas is recommended in the environmental vision of the Finnish Kennel Club strategy for 2003–2012 (SKL 2003). There would surely be a need for livestock guarding dogs, if knowledge about the dogs and the possibilities of using them would reach the people who need a trustable guard for their livestock or property (Koljonen 2002).

## OBJECTIVES

The goals of the study are to find the most cost-effective method of protecting livestock with LGD. In addition, the aim is to produce objective information for livestock owners and other stakeholders of breeds/breed lines and LGD behavioural qualities suitable for the Northern Europe and Baltic Sea Area conditions.

## METHODS

Phase 1 of the study included semi-structured interviews based on a questionnaire, in-site-visits to farms in the summer of 2006 and contacting stakeholders as well as theoretical reviewing. The media of recruiting the farms was through newspaper and web site announcements. Early LGD adopters were contacted and variables to be included in future studies were explored. The emphasis was on the socialization of the dogs and on estimating the pros and cons of using LGDs for guarding pastures typical to the target area conditions.

## EXPERIMENTAL DATA AND RESULTS

A total of 12 farms replied and of these 8 were included in the study. Selection criteria included that the farms actually used or have acquired their LGDs as working livestock guarding dogs, although exclusively full time working was not required. The total farm area varied between 2 and 77 ha (median 48,5 ha). Geographically 4 farms were located in traditional large carnivore areas in eastern and 4 in central parts of Finland. Of the farms one was located in the middle of the village, 5 farther from other houses and 2 in isolation in the middle of forest. Distances from the farms to neighbours varied between 0,02 and 6 km (median 0,3 km).

The number of residents on the farms was 29 in total, including 14 women and 15 men. The age range was 1 to 60 years (median 31) and included 10 children. Visitors or other people simply passing the farm depending of the season were e.g. neighbours, cyclists, mopedists, cars etc. passing via village roads. Because of the public right of access in surroundings of the farms can be also people going to pick berries, people jogging, skiers, snowmobilers, hunters or tourists.

Of the farms 7 kept sheep, 7 poultry, 1 dairy cattle, 1 beef cattle, 4 horses and 1 bees. The sample also included one horticulture farm, which was only starting to rear alpacas, currently owning one male and one female. The livestock breeds on the farms included Finn sheep (6 farms), Finn hens (6 farms) and Finn horses (3 farms). The LGDs' guarding areas thus included several kinds of fence types: electric fence (5), light electric fence (1), sheep fence (6), wolf fence (3), wooden fence (1) and no fence (2).

The total number of dogs was 31, varying between 2 and 7 per farm (median 4), from which 1 to 4 dogs were LGDs (median 2). Thus the total of LGDs was 19, of which 18 were working dogs. The number of different LGD breeds was 8 and included Caucasian Ovcharka, Central-Asian Ovcharka, Great Pyrenees, Komondor, Tibetan Mastiff, Maremma Sheepdog, Polish Tatra Sheepdog and Slovakian Cuvac. Of all the LGDs only 2 had been imported from abroad: one Komondor from Hungary and one Tibetan Mastiff from USA, while the rest have been bought from Finnish breeders. None of the LGDs had parents as working dogs. The farmers had acquired information about LGDs from books (5), breeders (4), internet (4), newspapers (3), pedigree dog association (2), the Finnish Kennel Club (1) and from another LGD owner (1). On all farms people had earlier experience of dogs and 4 had long term dog owner experience.

The main reason for acquiring the dogs were carnivore damages (2 farms) and continuous daily or weekly large carnivore observations (6 farms). Thus, on all every farms the residents had perceived danger of meeting large carnivores in their yard or in the neighbourhoods. The individual LGDs were chosen on the basis of gender or recommendation of the breeder, appearance and estimated character. However, only two puppies had been aptitude tested. The gender distribution of the dogs was 9 females, 9 males and 1 sterilized male.

According to the owners' estimations the guarding abilities first occurred at and age of 4 to 20 (median 12 months), depending on the breed and on the dog's personality. The season for bonding the LGDs to the livestock was spring (5), summer (6), autumn (1) and winter (6). The age for starting the bonding process varied from birth to 32 weeks (median 8 weeks). Bonding occurred mainly on pastures and partly in the sheep house with 4 LGDs. Owners answered to gain more excellent behaviour in relation to that amount they invested time or other effort for socialization process or human tolerance training.

In 3 farms guarding took place merely at nights. Only in one farm was a special high fence ought to stop dogs wandering, in other farms the fences did not hinder dogs passing trough. There were a few minor difficulties in inter-animal relationships (playing, chasing etc.). Herding dogs and LGDs got along acceptably.

The experiences of early LGD adopters were encouraging: all the farms that answered had gained from having LGDs and no-one reported damages after the dogs were introduced. The dogs had prevented some attacks or other damages.

Frequency table					
Characters of wild animal situations in numbers of farms.					
Wild animal	Observations	Meetings	Damages before LGDs	Estimated prevented damages	Damages when LGDs
Wolf	7	1	1	2	0
Bear	6	2	0	1	0
Lynx	5	0	1	1	0
Fox	2	1	0	0	0
Dog	1	0	1	0	0
Elk	3	3	2	2	0
White-tailed deer	1	1	1	1	0

Other benefits mentioned were the termination of elk damages to pasture fences and of damage by white-tailed deer to horticultural plants. In addition, the presence of LGDs had a more multifunctional character by increasing personal feelings of security in a comprehensive way. This included issues like how freely children can be permitted to be outdoors and feelings of companionship. LGDs also enhanced social networking, e.g. through so called coop-LGDs, aiming to help also neighbours with guarding. In the future, 7 of the farms are going to continue using LGDs; one dog was too young for evaluation. No LGD owner mentioned the price or maintenance costs as a disadvantage or problem.

The semi-structured questions focused on three main topics when considering possible benefits and disadvantages: people, dogs and general conditions on the farms. These first findings showed no direct disadvantages of LGDs. On the contrary, advantages mentioned were versatile. However the follow-up contacts during the year indicated some possible questions, regarding one dog not turning out as hoped (wandering too much) and one dog having a suboptimal relation to other animals (playing and chasing). As had been found before, a successful bonding with the stock the dog is going to protect is vital to successful guarding (Davydiak & Sims 2004).

## CONCLUSIONS

The aim of the semi-structured in-site visit study was to identify possibilities and problems associated with LGDs. The experiences of early LGD adopters were encouraging: all farms answered have gained from the dogs. The results to take account from this phase of the study were: the need for additional fencing seems not to be essential; various LGD breeds are possible, as well as it seem to be no obvious constraints on a certain species of livestock or other domestic animals to be guarded. LGDs guarding abilities could be used in varied way additionally to traditional full time guarding on pastures on open landscapes. Integrated use could also be part-time protecting and LGD's use together with wolf fences but also focusing to guarding farm property etc. at yards.

The puppy or adolescent time seems to be most important factor when sizing up the dogs guarding career. By means of puppy video-taped aptitude testing is possible to analyse and follow LGDs progress from the start. The two mild question cases showed however that there can be

problems to deal with when the using of LGDs increases. Also the relationships of traits like human tolerance and guarding behaviour of some LGD breeds may affect the selection and success of LGD. The coexistence of LGDs and herding dogs on the same farm is important as larger livestock herds can demand the use of both dog types.

Minimizing the carnivore damages or as importantly the fears of people is a multifunctional study topic. In summary the themes or factors were: dogs welfare in guarding job, people in and outside the farms, public opinion in nature relations, cost-effectiveness, cultural, socio-economic and stakeholder relations in general. Both tentative discussions and contacts from new LGD owners together are demonstrating spontaneous way to solve problems caused by carnivores. Stakeholder-work embodied also establishing good working relationships with local communities. For future research continuation the multidimensionality is to be emphasized.

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## PERFORMANCE AND BEHAVIOUR OF DAIRY BULLS RAISED AT PASTURE AND IN AN UNINSULATED BARN

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### SUMMARY

We compared performance, fatty acid profile of the meat, and behaviour of finishing dairy bulls raised at pasture and in an uninsulated barn. Grazing had no significant effect on the live weight gain, carcass conformation score or carcass fat score of the bulls. However, grazing improved polyunsaturated and saturated fatty acid ratio of the meat. Differences in the distribution of behaviours between the housing environments resulted mostly from the different feeding regimes and different space allowances. Stereotyped tongue-rolling was almost absent in both environment and there were no differences between the environments in time spent butting. This indicates that both housing environments were satisfactory in regard to the bulls' welfare.

**Keywords:** beef production, dairy bulls, grazing, growth, fatty acid profile, behaviour

### INTRODUCTION

In countries with long grazing season steers are commonly grazed in extensive beef production systems (e.g. Rueda et al. 2003). In Finland, grazing is strongly restricted by a short growing season and use of uncastrated animals. Grazing bulls is unusual because the bulls are considered as restless grazers and they may have impaired growth at pasture (Nisula and Hakkola 1979). However, grazing may have positive effects on meat quality and on the behaviour of bulls. We compared performance, fatty acid composition of the meat, and behaviour of finishing dairy bulls at pasture and in an uninsulated barn.

### MATERIAL AND METHODS

The experiment was conducted at the North Ostrobothnia Research Station of MTT Agrifood Research Finland in Ruukki (64°44'N, 25°15'E). Nineteen Finnish Ayrshire and Friesian bulls were used in the experiment. All animals were purchased as calves from local dairy farms in the spring 2003. They were kept at pasture (pasture bulls, see further) or in an uninsulated barn (barn bulls) during their first summer and in the uninsulated barn during the following winter. At the beginning of June 2004, the bulls (average age 15 months and weight 552 kg) were assigned to four groups of 4–5 animals. Two groups of bulls were housed in partly bedded pens (6.4 m<sup>2</sup>/bull) in the uninsulated barn and fed grass silage *ad libitum*. Two groups of bulls were turned to

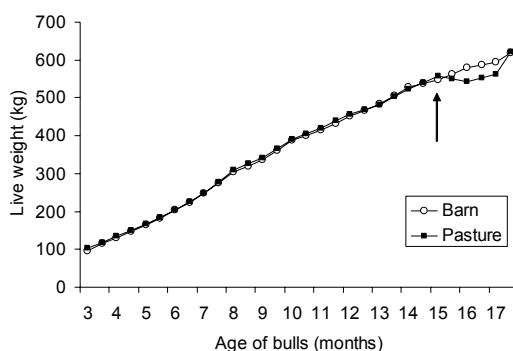
pasture. Both pasture groups were rotationally grazed on three perennial (timothy) and two annual (oat and Italian rye-grass mixture) paddocks (0.5 ha per paddock) with animals being moved to a new paddock on average once a week. Both pasture and barn bulls got barley 4.4 kg DM per animal per day. There was 0.7 m and 0.5–0.6 m feeding space per bull at the feeding trough in the barn and at pasture, respectively.

The behaviour of the bulls was observed for 24 hours in both June and July using instantaneous sampling method with a 6-min sampling interval. The pasture bulls were observed directly from an observation tower and the barn bulls were video recorded using a time-lapse video recorder. During both observations, the pasture bulls were at annual oat and Italian rye-grass mixture paddocks. The percentages of the observations spent on different behavioural patterns were tested with a linear mixed model. In the model, the housing environment and the month of summer were included as fixed effects and the group in the housing environment and the animal as random effects.

Grazing season extended 77 days (8.6.–23.8.2004) and after that both pasture and barn bulls were slaughtered. The live weight gain (LWG) was calculated as the difference between the means of initial and final live weights (LW). The carcasses were classified for conformation (scale from 1 to 15) and fat cover (scale from 1 to 5) using the EUROP quality classification. Fatty acid composition of the meat was measured from *Longissimus dorsi* muscle by gaschromatographic analysis (Metcalf and Schmitz 1961, Hara and Radin 1978). Animal performance data was subjected to analysis of variance using general linear models procedure.

## RESULTS AND DISCUSSION

Live weight data of the bulls before and during the grazing season are shown in Figure 1. There was no significant difference ( $P>0.05$ ) in the LWG (average 890 g/d) between the barn and pasture bulls during the grazing season. However, during the first grazing weeks, pasture bulls lost a considerable amount of weight (Figure 1). Similar live weight losses have been reported also for steers at the beginning of the grazing season (McCarrick and Drennan 1972, Scollan et al. 2001). Tayler et al. (1957) have showed that most of this kind of weight loss is gut fill, associated with changes in diet digestibility and intakes.



**Figure 1.** Live weights of dairy bulls housed in the barn and at pasture. The arrow indicates turnout to grazing of the pasture bulls.

**Table 1.** Fatty acid profiles (g/kg of total fatty acids) (mean  $\pm$  SD) of in *Longissimus dorsi* muscle of dairy bulls housed in barn and at pasture.

Fatty acid	Barn	Pasture	Effect
14:0	22.6 $\pm$ 6.1	16.3 $\pm$ 2.9	*
14:1 <i>n</i> -5	4.7 $\pm$ 2.5	3.0 $\pm$ 0.8	
15:0	2.0 $\pm$ 0.4	1.7 $\pm$ 0.4	
16:0	238.0 $\pm$ 8.5	227.9 $\pm$ 11.2	*
16:1 <i>n</i> -7	31.3 $\pm$ 9.7	25.3 $\pm$ 11.2	
17:0	6.9 $\pm$ 0.9	6.0 $\pm$ 1.4	
17:1	5.1 $\pm$ 1.0	4.3 $\pm$ 1.2	
18:0	160.5 $\pm$ 9.0	153.0 $\pm$ 21.2	
18:1 <i>n</i> -7	14.0 $\pm$ 1.5	16.2 $\pm$ 2.1	*
18:1 <i>n</i> -9	424.0 $\pm$ 23.0	423.4 $\pm$ 18.7	
18:2 <i>n</i> -6	39.6 $\pm$ 12.3	59.7 $\pm$ 19.1	*
18:2 <i>cis</i> -9, <i>trans</i> -11 CLA	1.1 $\pm$ 0.4	1.2 $\pm$ 0.6	
18:3 <i>n</i> -3	6.9 $\pm$ 1.6	7.8 $\pm$ 2.1	
20:0	0.8 $\pm$ 0.2	0.7 $\pm$ 0.2	
20:1 <i>n</i> -9	1.8 $\pm$ 1.0	1.8 $\pm$ 0.7	
20:2 <i>n</i> -6	0.2 $\pm$ 0.5	0.0 $\pm$ 0.0	
20:4 <i>n</i> -6	9.0 $\pm$ 3.5	15.6 $\pm$ 7.6	*
20:5 <i>n</i> -3	0.5 $\pm$ 0.5	1.3 $\pm$ 1.0	*
22:5 <i>n</i> -3	1.5 $\pm$ 0.8	2.8 $\pm$ 1.9	
Unidentified fatty acids	29.7 $\pm$ 5.5	31.8 $\pm$ 4.8	
SFA <sup>1</sup>	430.7 $\pm$ 12.8	405.6 $\pm$ 28.2	*
MUFA <sup>2</sup>	481.0 $\pm$ 22.6	474.2 $\pm$ 23.8	
PUFA <sup>3</sup>	58.6 $\pm$ 16.6	88.4 $\pm$ 30.6	*

<sup>1</sup> Saturated fatty acids; <sup>2</sup> monounsaturated fatty acids; <sup>3</sup> polyunsaturated fatty acids.

\* P<0.05.

There were no significant effects (P>0.05) of housing environment on the carcass weight (average 329 kg), carcass conformation score (4.8) or carcass fat score (2.1). Compared to barn-housing, grazing increased proportion of 18:1 *n*-7, 18:2 *n*-6, 20:4 *n*-6 and 20:5 *n*-3 fatty acids and decreased proportion of 14:0 and 16:0 fatty acids in *Longissimus dorsi* muscle (Table 1). There were no significant differences between the barn and pasture bulls in the content of *cis*-9, *trans*-11 CLA in the meat. However, there was a higher content of polyunsaturated fatty acids (PUFA) and lower content of saturated fatty acids in the meat of the pasture bulls than of the barn bulls. Also according to French et al. (2000) grazed grass was an effective diet for elevating PUFA content of meat.

Due to the different feeding regimes the barn bulls were observed to spend more time eating at the feeding trough than the pasture bulls in June and July (Table 2). Only barley was offered in the feeding trough to the pasture bulls, and, consequently, they spent a lot of time foraging grass in the paddocks. Pasture bulls were observed grazing and ruminating less in June than in July. Possibly, as the summer advanced the increase in fibre content and decrease in digestibility of the oat and Italian rye-grass mixture affected the time spent grazing and ruminating in the pasture bulls.

There was no difference between the groups in time spent licking and biting (manipulating) objects and structures of the environment (Table 2). In our study, this behaviour seemed to be

mostly normal investigative behaviour, since it had only little or none stereotyped features. There was no difference in self-grooming between the groups. The higher proportion of walking in the pasture bulls compared to the barn bulls was probably a natural consequence of the larger living area in the pasture. Walking during grazing was not taken into account in our study, and therefore the pasture bulls were actually moving even more than the current results indicate. Daily exercise promotes health and agility in tethered cows (Gustafson 1993, Gustafson and Lund-Magnussen 1996), and it is reasonable to assume that exercise has positive effects also on the health of bulls.

**Table 2.** Percentage of observations (mean  $\pm$  SD) spent on different behavioural patterns in June and July in bulls housed in barn and at pasture. P1: significance between housing environments, P2: significance within housing environments between months, P3 significance of interactive effect of housing environment and month.

		Barn	Pasture	P1
Eating silage or barley at the feeding trough	June	11.4 $\pm$ 2.0	2.3 $\pm$ 0.6	***
	July	11.7 $\pm$ 2.7	2.1 $\pm$ 1.1	***
	P2			P3
Grazing	June	–	13.8 $\pm$ 3.7	–
	July	–	15.9 $\pm$ 4.5	–
	P2		*	–
Ruminating	June	33.9 $\pm$ 3.5	22.7 $\pm$ 5.3	***
	July	33.7 $\pm$ 2.7	30.6 $\pm$ 5.2	
	P2		**	P3 **
Manipulating objects with mouth or tongue	June	1.5 $\pm$ 1.8	0.44 $\pm$ 0.43	
	July	1.9 $\pm$ 1.3	0.19 $\pm$ 0.31	
	P2			P3
Self-grooming	June	2.1 $\pm$ 0.8	1.9 $\pm$ 1.7	
	July	2.3 $\pm$ 1.1	2.8 $\pm$ 2.3	
	P2			P3
Walking excluding walking during grazing	June	1.4 $\pm$ 0.6	2.5 $\pm$ 0.7	**
	July	1.5 $\pm$ 0.8	3.0 $\pm$ 0.8	***
	P2			P3
Social licking	June	2.4 $\pm$ 1.0	2.3 $\pm$ 1.3	
	July	1.3 $\pm$ 0.9	1.2 $\pm$ 1.3	
	P2	*		P3
Butting	June	4.0 $\pm$ 1.6	4.5 $\pm$ 1.6	
	July	3.6 $\pm$ 2.4	3.7 $\pm$ 1.5	
	P2			P3
Lying inactive or resting	June	28.2 $\pm$ 5.2	32.6 $\pm$ 5.9	
	July	27.7 $\pm$ 4.5	29.2 $\pm$ 4.5	
	P2			P3
Tongue-rolling	June	0.21 $\pm$ 0.30	0.0 $\pm$ 0.0	<sup>2</sup>
	July	0.08 $\pm$ 0.18	0.0 $\pm$ 0.0	<sup>2</sup>
	P2	<sup>3</sup>	<sup>3</sup>	–
Other behaviours e.g. idling in standing position	June	15.5 $\pm$ 3.6	17.3 $\pm$ 6.4	
	July	16.6 $\pm$ 3.4	11.7 $\pm$ 5.4	
	P2		***	P3 **

Since the residuals of the variable tongue-rolling were not normally distributed, the variable was tested with nonparametric testes: <sup>2</sup>Mann-Whitney test; <sup>3</sup>Wilcoxon test.

\* P<0.05; \*\* P<0.01; \*\*\* P<0.001.

Social behaviour was very similar in the barn and pasture bulls and there were no differences in social licking or butting between the groups (Table 2). This result is contradictory with other studies where a decrease in the space allowance has increased agonistic interactions in female cattle and steers (Kondo et al. 1989), and accordingly, in cows, agonistic interactions have been observed more inside cubicle houses than at pasture (O'Connell et al. 1989). Bull calves are socially more active than female calves (Reinhardt et al. 1978), and since the animals were quite young in our study, bulls' keenness for social contact may explain similar proportions of butting found in the barn and the pasture bulls. Furthermore, in both groups butting behaviour seemed to be mostly fairly harmless and often resembled mock fighting (see Reinhardt and Reinhardt 1982).

There were no differences in time spent on resting or performing other behaviours (e.g. idling in standing position) between the barn and pasture bulls (Table 2). Stereotyped tongue-rolling was very rarely observed in the bulls. Stereotyped behaviour in cattle is often associated with long-lasting frustrating situations such as restricted feeding (Redbo and Nordblad 1997) or tethering (Redbo 1992). Accordingly, this may suggest that in our study, the barn and pasture bulls were not subjected to strong long-lasting frustration, since stereotyped tongue-rolling and other stereotypies were almost absent.

## CONCLUSIONS

Grazing had no effect on animal performance or carcass conformation but it improved polyunsaturated and saturated fatty acid ratio of the meat, and made it more compatible with consumer requirements. Differences in the distribution of behaviours between the housing environments resulted mostly from the different feeding regimes and different space allowances. Stereotyped tongue-rolling was almost absent in both environment and there were no differences between the environments in time spent butting. This indicates that both housing environments were satisfactory in regard to the bulls' welfare.

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**A2 –  
IMPACT OF ENVIRONMENT ON PIGS' BEHAVIOUR,  
HEALTH AND PERFORMANCE**





## ORAL PRESENTATIONS

### EPIDEMIOLOGICAL ANALYSIS AND CLASSIFICATION OF THE HEALTH STATUS OF PIG HERDS – THE HERD HEALTH INDEX (HHI)

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#### SUMMARY

Quantifying the herd health of food producing animals is a major precondition for improving the husbandry systems towards better health and welfare conditions. For assessing the health status of fattening pig herds accurately, multiple recurrent clinical examinations during the entire fattening period seem to be irreplaceable. Since this is, however, impossible in the routine production, indirect measurements of the herd health are needed. Therefore, a Herd Health Index (HHI) based on retrospective data was developed. First results of testing the HHI as tool for quantifying the health of pigs from pig-fattening farms are presented.

**Keywords:** Benchmarking animal health and animal welfare, Animal Treatment Index (ATI), Herd Health Index (HHI)

#### OBJECTIVE

The Reg. (EC) No. 178/2002 demands for the inclusion of the primary production into the food safety system and asks for a risk-based approach to food safety. Regarding the fact that data on the health status of slaughter animals has to be a part of the food chain information, it is essential to be able to quantify the health of food animal herds.

The objective is to develop, evaluate and validate a robust tool for quantifying the health status of pig herds and batches of slaughter pigs to serve as decision making tool for the risk-based meat inspection, but also for other risk-based decisions such as targeted residue testing and benchmark systems for creating incentives for the improvement of animal health and welfare of food producing animals. The paper describes the development of a Herd Health Index (HHI) and its testing by comparing the HHI of 20 herds with an intensive repeated clinical examination of the 20 herds by one veterinarian.

#### MATERIAL AND METHODS

For this study, various data reflecting the health of fattening pigs (representing grow-finish to slaughter) from 20 farms of a cooperative were gathered at least 6 times per group during the whole grow-finish and finish period. This cooperative is situated in Northern Germany and is

composed of 440 pig-fattening farms, which get their piglets without exception from an associated piglet rearing cooperative, i.e. all farmers work with the same genetics. The slaughter pigs are slaughtered at the same abattoir which also belongs to the cooperative. This horizontally and vertically quite well coordinated structure provides the basis for a harmonised data recording. On this basis, a comparison of the results of an intensive clinical investigation to an indirect measuring of the health status using the Herd Health Index (HHI) was carried out.

### CLINICAL INVESTIGATIONS INDEX

All fattening-groups of pigs in the 20 study herds were clinically examined at least 6 times evenly distributed over the whole fattening-period by strictly one person (first author). Out of 15 measured health indicators the following 6 criteria turned out to be most suitable for a semi-quantitative estimation of the health of the examined pig groups:

- a) The incidence and duration of **respiratory diseases** (0 to 4 points),
- b) The incidence and duration of **diarrhoea** (0 to 2 points),
- c) The incidence and duration of **limb-lesions** (0 to 2 points),
- d) The incidence and duration of **skin-diseases** (0 to 2 points),
- e) The incidence and duration of **cannibalism** (0 to 2 points), and
- f) The **evenness of weight gain within the pig group** (0 to 2 points).

Each of the 6 examinations resulted in additive “health points”, which range from 0 to 14 points. In order to represent the animal health status during the whole fattening period, the 6 examinations were spread throughout the entire finishing period (2 in the first, two in the second and two in the last third of the fattening period). At the time of slaughtering the slaughter pig batches of a finishing group, the health points of the 6 examinations were combined to one cumulative “batch health point” (= the average of the six health points). The batch health points of one herd were again combined to a “herd health point”, which then was the criterion that was compared to the HHI of the herd in question. To use the herd health points for a classification system, the following classes were created: 0 to 3 health points is score 0, 4 to 6 is score 1, 7 to 10 is score 2, and > 10 health points is score 3.

### THE COMPOSITION OF THE HERD HEALTH INDEX (HHI)

The following 4 parameters were gathered, rated, and combined to the HHI:

- 1) The **mortality rate**,
- 2) The **frequency of pathological findings in carcasses** of previous meat-inspections,
- 3) The **animal-treatment-index (ATI)**, and
- 4) The **duration of the fattening-period**.

#### 1) Mortality rate

If the mortality rate of a fattening group did not exceed 2% at the end of the fattening period this parameter was rated as mortality score 0. A frequency between 3% and 5% resulted in score 1, between 5% and 10% in score 2, and if the percentage of deceased pigs exceeded 10% the mortality score was 3.

## 2) Herd prevalence of pathological findings during previous meat-inspections

The frequency of gross pathological lesions found in carcasses and the organs of slaughter pigs of a herd is a quite objective indicator of the occurrence and severity of most diseases in pigs. Based on the Organ-Lesion-Index according to BLAHA (1994) the diagnostic findings of the meat-inspections of the slaughter pigs of each pig farm in the last six months were summarized in an index which varies from 0 to 10. Thereby the frequencies of pleurisy, pneumonia, liver-lesions and pericarditis per slaughter pig batch, and per herd (= cumulative index from consecutive batch) were rated and summarized in values from 0 to 10.

Organ-Lesion-Index according to BLAHA (1994)

<b>pleurisy</b>	frequency	<1%	1–10%	11–30%	>30%
	points	0	1	2	3
<b>pneumonia</b>	frequency	<1%	1–10%	11–30%	>30%
	points	0	1	2	3
<b>liver lesions</b>	frequency	<1%	1–10%	11–30%	>30%
	points	0	1	2	3
<b>pericarditis</b>	frequency	<1%	≥ 1%		
	points	0	1		

For calculating the HHI, a “Blaha-Index” less than 2 results in a lesion frequency score of 0, values from 3 to 5 were rated as score 1, points between 6 and 8 resulted in score 2, and a “Blaha-Index” higher than 8 leads to a lesion frequency score of 3.

## 3) Animal-Treatment-Index (ATI):

The Animal-Treatment-Index (Blaha et al., 2006) stands for the average frequency of medicating every pig in the group with antimicrobial substances, based upon the hypothesis that healthy pigs get less medication than pigs with a poor health and clinical illness. The formula is:

$$\text{ATI} = \frac{\text{No. of animals treated} \times \text{No. of treatment days}}{\text{Total} - \text{No. of animals in the herd or group}}$$

If the ATI does not exceed 10 days of medicating, this ends up in 0 points. An ATI between 11 and 20 days results in an ATI score of 1, ATI-values from 20 to 40 lead to an ATI score of 2. If a fattening group of pigs has been medicated with antimicrobial substances more than 40 days, the ATI score is 3.

## 4) Duration of the fattening period

On the study farms, all farmers start fattening piglets with a body weight between 27 and 31 kg and intend to send the slaughter pigs to abattoir with a body weight of 115kg to 121kg (= window of best price). If the time for fattening a batch of slaughter-pigs is less than 100 days this is calculated with score 0. A fattening duration period between 100 and 120 days results in score 1, a

duration from 121 to 150 days accounts for score 2, and if the fattening took more than 150 days this is taken into account with score 3.

**The HHI:** In the end the sum of the 4 single scores is added to the Herd-Health-Index (HHI) which can vary between 0 and 12 points. The HHI can be calculated for single batches, but also for herds for whatever period of time.

## RESULTS

Although the farmers in the investigated system use the same genetics, and are to follow the same guidelines for their herd health management, the single HHI's vary a lot. Some farmers fatten their pigs up to 120 kg within 91 days, without any losses and extremely little amounts of antibiotics. Other farmers loose 12,24% of their pigs, fattening their pigs takes more than 150 days and by treating them with antibiotics for more than 4 weeks. In most cases, the overall organ lesion frequency of slaughter batches from one farm is repeatedly the same over time, but there is no single parameter on its own which allows a reliable prediction about the health status of slaughter pig batches.

Tables 2 and 3 demonstrate as examples the relation between HHI and clinical findings.

**Table 2.** Herd health criteria and the HHI of Farm A

Batch-No.	1	2	3	4	5	6	7	8	9	10
Mortality (%)	6,25 → 2	6,25	6,25	6,25	12,24	12,24	12,24	12,24	12,24	12,24
Score		→ 2	→ 2	→ 2	→ 3	→ 3	→ 3	→ 3	→ 3	→ 3
Blaha-Index	6	6	6	6	6	6	6	6	6	6
Score	→ 2	→ 2	→ 2	→ 2	→ 2	→ 2	→ 2	→ 2	→ 2	→ 2
ATI (d/pig)	52,05 → 3	52,05	52,05	52,05	45,15	45,15	45,15 %	45,15	45,15	45,15
Score		→ 3	→ 3	→ 3	→ 3	→ 3	→ 3	→ 3	→ 3	→ 3
Duration of fattening (d)	138	146	154	163	100	112	121	129	142	149
Score	→ 2	→ 2	→ 3	→ 3	→ 1	→ 1	→ 2	→ 2	→ 2	→ 2
<b>HHI</b>	<b>9</b>	<b>9</b>	<b>10</b>	<b>10</b>	<b>9</b>	<b>9</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>
<b>Clinical examination score</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>2</b>

The HHI of Farm A (average of batches 1 to 10) = 10. The average clinical examination score of Farm A = 2

**Table 3.** Herd health criteria and the HHI of Farm B

Batch-No.	1	2	3	4	5	6	7	8	9
Mortality (%)	0	0	0	0	0	2,11	2,11	2,11	2,11
Score	→ 0	→ 0	→ 0	→ 0	→ 0	→ 0	→ 0	→ 0	→ 0
BLAHA-Index	1	1	1	1	1	2	4	4	4
Score	→ 0	→ 0	→ 0	→ 0	→ 0	→ 0	→ 1	→ 1	→ 1
ATI	0	0	0	0	0	0,01	0,01	0,01	0,01
Score	→ 0	→ 0	→ 0	→ 0	→ 0	→ 0	→ 0	→ 0	→ 0
Duration of fattening (d)	91	97	102	118	124	95	102	111	125
Score	→ 0	→ 0	→ 1	→ 1	→ 2	→ 0	→ 1	→ 1	→ 2
<b>HHI</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>0</b>	<b>2</b>	<b>2</b>	<b>3</b>
<b>Clinical examination score</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>

The HHI of Farm B (average of batches 1 to 10) = 1. The average clinical examination score of Farm B = 0

Table 4 demonstrates the composition of the HHI's of all study farms. The scores of the Farms C to T were calculated as shown for Farms A and be (cf. Table 2 and 3)

**Table 4.** The Herd-Health-Index (HHI) compared to the clinical findings of all 20 study farms

farm	mortality score	ATI	Blaha-Index score	duration of fattening score	HHI	clinical examination score
A	3	3	2	2	<b>10</b>	2
B	0	0	0	1	<b>1</b>	0
C	1	0	1	2	<b>4</b>	1
D	0	1	0	1	<b>2</b>	0
E	1	1	1	2	<b>5</b>	1
F	2	0	1	1	<b>4</b>	1
G	2	1	1	2	<b>6</b>	0
H	0	1	1	2	<b>4</b>	1
I	0	1	1	2	<b>4</b>	0
J	1	1	1	3	<b>6</b>	0
K	1	1	1	1	<b>4</b>	0
L	0	0	1	1	<b>2</b>	1
M	1	1	1	2	<b>5</b>	1
N	1	0	0	1	<b>2</b>	0
O	1	2	1	2	<b>8</b>	2
P	1	0	1	1	<b>3</b>	0
Q	1	1	0	1	<b>3</b>	1
R	1	1	1	1	<b>4</b>	1
S	2	0	2	2	<b>6</b>	1

## DISCUSSION AND CONCLUSION

The chosen criteria for indirectly measuring the health of pig groups (slaughter batches, finishing groups and/or herds) seem to provide a rather accurate reflection of the health history of pigs sent to slaughter. Calculating a Herd Health Index supports greatly the estimation of the herd health of slaughter pigs, which can be used for the risk-based meat inspection, for benchmarking the herd health status of herds supplying the same slaughter house (as tool for the improvement of animal health and animal welfare), and for other risk-based decisions such as targeted residue testing. In further investigations the HHI should be validated in other production systems.

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## **RISK-BASED MEAT INSPECTION AND RISK-BASED OFFICIAL CONTROLS AS MEANS FOR IMPROVING ANIMAL HEALTH AND ANIMAL WELFARE**

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### **SUMMARY**

The paper describes the need and ways of the transition from the traditional meat inspection being an end product inspection to a risk-based meat inspection being a process optimisation throughout the food chain as required by the new EU food safety regulations. Concluding from many data analyses at German slaughter houses with their supplying pig herds, criteria and an evaluation system for how to use these criteria in the framework of the “relevant food chain information” and the risk-based meat inspection are described and discussed. Finally, it is described how relevant parts of the food chain information can (and must) be used as basis for benchmarking the animal health and welfare as tool for a continuous improvement process in managing pig herds.

**Keywords:** risk-based meat inspection, food chain information, benchmarking animal health and animal welfare

### **INTRODUCTION**

The recent crises in the meat industry due to meat-associated risks such as salmonella, nitrofen and dioxin prove that the traditional ante- and post-mortem inspection of slaughter animals and carcasses is not any longer able to recognise and prevent the risks of today. Therefore, the EU Commission has issued Reg. (EC) 853/2004 and Reg. (EC) 854/2004 that regulate the transition of the traditional meat inspection, which demands inspecting each individual carcass in the same way, to a risk-based meat inspection, which is using relevant pieces of information about the previous production stages for making risk-based decisions on the intensity of the inspection of slaughter pig batches. The present paper is describing the legal framework and the objectives of the risk-based meat inspection, and how the food chain information for the risk-based ante- and post-mortem meat inspection can and should be used for improving animal health and welfare.

### **OBJECTIVE**

The Regulations (EC) No 853/2004 and (EC) 854/2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption describe the risk-based approach for the official food supervision. The new EU legislation is not any longer prescribing exactly the inspection procedure, but defines the food safety goals. The consequence is that there are still various ideas and opinions on how to implement a reasonable risk-based meat inspection, and, in particular, on how to design the “relevant food chain information” a) for the decisions to be made in the framework of the risk-based meat inspection,



and b) for a reasonable feed-back to the farmer and his veterinarian as basis for improvements at farm level. The food chain information has to be designed in a way that it is possible for the official veterinarian to estimate the probability that slaughter pigs carry a higher or lower risk to human health and the frequency of lesions that has to be expected. Depending on the estimated risk for the consumers (e.g. zoonotic infections, residues, parasites and microbiological contamination) and the expected frequency of lesions, the official veterinarian has to decide for announced slaughter pig batches, whether the meat inspection of the carcasses has to be “visual”, “traditional” or “more intensive”. This means that the carcasses of slaughter pig batches, for which the food safety risk is estimated as low and the expected lesion frequency is low, can be inspected without palpation and incisions (= visual inspection). If the risk level is estimated as medium and/or the expected lesion frequency is high, the decision of the official veterinarian has to be that the carcasses are to be inspected in the current way with palpation and incisions including the removal of pathologically altered organs (= traditional inspection). In those cases, where the food chain information signals a high food safety risk and/or extremely high frequencies of lesions, the decision must be that special additional investigations tailored to the risk in question have to be applied (= more intensive inspection).

The objective of our research is to test data from the primary production that are available (and/or need to be generated) for their usability as basis for the creation of the “relevant food chain information”, which is not only serving the decision making process of the official veterinarian in the slaughter plant, but also the farmer and his veterinarian as basis for a continuous improvement of the herd health status and the animal welfare.

## MATERIAL AND METHODS

The following steps were taken to reach the objective:

- a) analysing the complex legislative texts that regulate the transition of the current meat inspection procedure into the risk-based inspection system (instructions relevant for the risk-based meat inspection including the food chain information are embedded in at least four EU-Regulations: Reg. No. (EC) 178/2002, 852/2004, 853/2004, and 854/2004);
- b) analysing various sets of routinely generated food chain data (several slaughter plants with their suppliers) that are available from farm records, veterinary reports and the slaughter plant reporting system as well as developing and validating a new index for semi-quantifying the health status of finishing pigs;
- c) developing a proposal for meaningful food chain information usable for the decisions on the meat inspection intensity and for benchmarking the health status of pig herds as basis for improvements of the animal health and welfare.

## RESULTS

### *Legislation*

The food business operator (= the slaughter plant) has to organise that he is provided with the relevant food chain information by the farmer on each slaughter pig batch that the farmer is offering him for slaughter. The slaughter house operator is also responsible to make this food chain information available to the official veterinarian at least 24 hours before the arrival of the animals. The contents and form of this food chain information has to be in a way that enables the

official veterinarian to make an “informed” decision on the intensity of the meat inspection that has to be applied for the pig batch in question. This means that the official veterinarian must be able to answer the following questions:

- 1) Do the animals come from a holding that is an “integrated system”, and does the husbandry system fulfil the criteria of “controlled housing condition”?
- 2) Are the animal health status of holding of provenance and/or the regional animal health status indicating any severe disease?
- 3) What is (has been) the animals` health status?
- 4) Were veterinary medicinal products or other treatments with a withdrawal period greater than zero administered to the animals within a relevant period of time? Are dates of administration and withdrawal periods reported?
- 5) Did diseases occur that may affect the safety of meat?
- 6) Are there diagnostic results relevant to the protection of public health including samples taken in the framework of the monitoring and control of zoonoses and residues?
- 7) Do reports about previous ante- and post-mortem inspections of animals from the same holding of provenance, in particular reports from the official veterinarian, indicate a repeatedly low or a repeatedly high frequency of pathological lesions?
- 8) Are there production data that might indicate the presence of disease during the fattening period of the slaughter pigs?
- 9) Is the name and address of the private veterinarian normally attending the holding of provenance available?

If the official veterinarian can answer these 9 questions, he or she should be able to predict the risk level and the frequency of lesions, which leads to the decision: “visual”, “traditional” or “more intensive” meat inspection.

#### ***Available data***

**Ad 1)** If the farm in question has a steady supply relation with the slaughter house and/or a reliable information flow between the farm and the slaughter house exists; the relationship can be defined as an “integrated system”. If the animals are routinely kept in a confinement system with daily attention and a reliable systematic recording system, the housing conditions can be defined as “controlled”. If there is a special standing arrangement with the slaughter house, or if the farmer is participating in a quality assurance scheme, this information is needed only once until changes have to be reported. Animals from ***“non-integrated” systems and “uncontrolled” housing conditions are to be excluded from the visual inspection.***

**Ad 2)** Information on diseases in the area or in the holding of provenance (potentially influencing food safety and/or notifiable diseases) should be available from official disease recording systems (international and/ or national) and taken into consideration when slaughter pigs are announced for slaughter.

**Ad 3) and Ad 7)** The following criteria offer the opportunity of estimating the health of a pig herd (finishing group):

- the mortality rate,
- the amount of antibiotic substances used for therapeutic and metaphylactic purposes, and
- the frequency of lesions in organs and carcasses from previous shipments from the same herd.

Mortality: Our results have shown that only very high death losses during the finishing period (> 5%) indicate severe animal health problems potentially leading to food safety risks and/or to a very high frequency of lesions in the slaughter pigs stemming from this herd. Animals from herds (finishing groups) with **mortality rates > 5% should be excluded from the visual inspection.**

The use of antibiotic substances: We (Blaha et al., 2006) developed the “simple” indirect indicator for animal health, the “Animal Treatment Index” (ATI):

$$\text{ATI} = \frac{\text{Number of treated animals X Number of treatment days}}{\text{Number of animals in the group}}$$

The ATI indicates the statistical number of days on which all animals of a herd or a group of pigs were treated with an antimicrobial substance. The basic idea is that animals are in the majority of all cases only treated for two reasons: a) disease (then the use of the antimicrobials is “therapeutic”), and b) the justified assumption of an infection, which will undoubtedly lead to disease (then the use of the antimicrobial is “metaphylactic”).

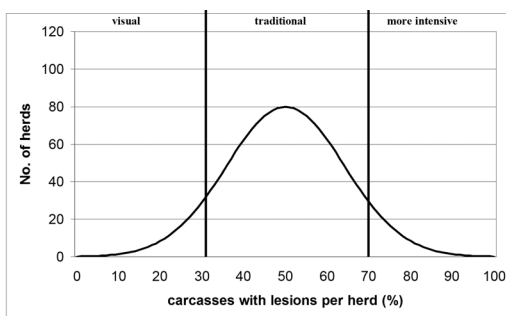
Our results of collecting data on the use of antibiotics in a group of 20 farms delivering pigs to the same slaughter house, and consecutively calculating the ATI for each of the finisher groups from which slaughter pig batches came from, led to the astonishing outcome that the ATI’s of about 200 slaughter pig batches varied between 0 and > 70 (!!). An ATI of 70 means that all pigs of the finisher group in question have been treated with antimicrobial substances over a period of time that covers about two thirds of the entire life span of these pigs (slaughtered approximately on day 110). Since an ATI of 30 means that all pigs of the group were treated with antimicrobial substances almost one third of the pigs’ life (in the light of public health concerns quite high), it should be considered to **exclude slaughter pig groups with an ATI > 30 from the visual inspection.**

The ATI and its potential use e.g. for a risk-based sampling scheme for monitoring systems for antimicrobial residues is explained in more detail in the paper by Dickhaus et al. (2007) published in these proceedings as well.

The frequency of pathological lesions in organs and carcasses from animals supplied to a slaughter house: Our manifold investigations of data collected on many slaughter houses in Germany show that the frequency of disease-related lesions is very consistent in animals from the same herd. This means that mostly, farmers that supply animals with lots of pathological lesions will be delivering animals with many lesions (unless they drastically change their management procedures), and, vice versa, farmers that deliver animals with only few lesions will be delivering animals with few lesions in the following shipments as well. This consistency provides the opportunity to record the lesion frequency of animals that had already shipped from the herds that supply a slaughter house (e.g. in the previous 6 months) and to categorise the herds according to their lesion frequency into e.g. “very few animals with lesions”, “an average number of animals with lesions”, and “many animals with lesions”. Following the logic of our so far explained approach to make a decision on the intensity of the meat inspection for slaughter pig batches, those herds that are in category **“very few animals with lesions” can be assigned to “visual inspection”, those that are as in category “an average number of animals with lesions” to “traditional inspection”, and those that are in category “many animals with lesions” to “more intensive inspection”.**

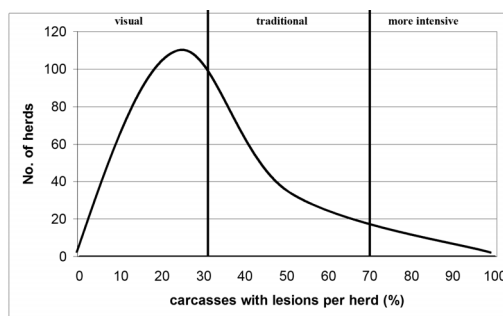
Figure 1 demonstrates how many herds were assigned to the three levels of intensity of the meat inspection in case the frequency of lesions per herd would be normally distributed.

Figure 2 shows how determining the real distribution of the herd-related slaughter check and meat inspection data can easily lead to a tool for the decision making process for the risk-based meat inspection.



**Figure 1.** Theoretical distribution of carcasses with lesions per herd

(The assumption: lesions are normally distributed)



**Figure 2.** Real distribution of carcasses with lesions per herd

(The reality: data of several German slaughter houses indicate a non-normal distribution)

**Ad 4)** The food business operator should negotiate with the competent authority and the supplying farmers how long the “relevant period of time” (Reg. 853/2004) is to be (e.g. the longest withdrawal time, two or three times the longest withdrawal time, or just 40 or 60 days...). The food chain information can easily contain a paragraph, assuring that this period of time without antibiotic treatments has been complied with. In cases with this assurance, but very high ATI’s, the visual inspection should be excluded and targeted samplings for residue testing may be initiated.

**Ad 5)** Since farmers cannot be expected to know and/or to notify any diseases that “may effect the safety of meat” (Reg. 853/2004), the already discussed indirect criteria such as mortality, herd prevalence of lesions at slaughter and the ATI (cf. Ad 3 and 7) should serve as possible indicators for disease and “trigger” targeted investigations into the herd health of herds with excessively high mortality rates, lesions in carcasses at slaughter and ATI’s e.g. higher than 30.

**Ad 6)** In the light of the Dir. 2003/99/EC and the Reg. (EC) 2160/2003, there will be an increasing impact of monitoring schemes for control of zoonotic pathogens in the EU member states and are to be used in the framework of the risk-based meat inspection. Monitoring schemes for the Salmonella control in poultry and pig production already exist in a growing number of states. Other monitoring schemes for e.g. *Campylobacter* and *Mycobacteria* are being developed, validated, and implemented. It is a must to take into consideration (= adding to the food chain) the results of any available monitoring scheme as well as any laboratory result pointing to zoonotic infections and/or residues in animals from the herds in question.

**Ad 8)** Except of using the data on the mortality rate, it is necessary to add to the food chain information any production data that are extremely out of the herd-specific range of variation (e.g. a fattening period of > 150 days in a herd with regularly 100 days, or > 200 in a herd with

regularly 150 days). However, a herd with always *fattening periods of e.g. > 150 should be excluded from the visual inspection* anyway.

**Ad 9)** The private veterinarian should be accessible any time by the official veterinarian in case a decision needs more information on the herd in question.

## DISCUSSION AND CONCLUSIONS

It is of utmost importance to underline that the threshold values for the mortality, the ATI and the frequency of lesions in carcasses and organs per herd are “food chain specific”. This means that every slaughter house operator has to calculate and regularly re-evaluate the real distribution of the herd prevalence of lesions at “his” slaughter house with its set of supplying pig herds. Once the threshold values are set for a slaughter house with its set of suppliers (e.g. as in Fig. 2), the following decision tool will be available:

1. all three criteria (mortality, ATI and frequency of lesions) of the finishing group, from which a slaughter pig batch comes from, are below the threshold values between “visual” and “traditional”: the decision for the meat inspection **can be visual inspection**;
2. one, two or all three criteria are above the threshold values between “visual” and “traditional”, but below the threshold values between “traditional” and “more intensive”: the decision for the meat inspection **should at least be traditional inspection**;
3. one or more of the three criteria is above the threshold value between “traditional” and “more intensive”: the decision for the meat inspection **must be more intensive inspection**.

A clear request of the Reg. (EC) No. 854/2004 is to use the food chain information additionally to its use for the risk-based meat inspection also for a process of continuous improvement of the animal health status of slaughter pig producing herds. Combining the three criteria into a “Herd Health Score” (Dickhaus et al., 2007) provides for a benchmarking tool for pig herds (at least of those herds that deliver pigs to the same slaughter house), which in turn is a basis for a targeted consulting for improvement measures in the herds with a high “Herd Health Score” (HHS). The HHS does not lead to a “diagnosis”, but it indicates that a herd has a problem and it already points to “problem areas”. The detailed analysis of the reasons for the suboptimal score and, if applicable, the diagnosis of underlying diseases must then be done on the farm in question. Thus, the creative use of specific parts of the relevant food chain information for not only improving the safety of the produced food, but also for improving the health and welfare of the animals kept for food production.

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## RELATIONSHIPS BETWEEN LYSINE LEVEL IN FEED OF WEANERS, ACUTE PHASE PROTEIN AND PERFORMANCES

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### ABSTRACT

Close relationships between nutrition, sanitary conditions and immune response have been described. Haptoglobin is an acute phase protein in pig. This biomarker has been shown to be linked to the sanitary conditions of pig farms. The objective of this work is to point out a possible relationship between the characteristics of feed during the post-weaning period and the level of haptoglobin in pig.

We evaluated in three trials the haptoglobin in blood of pigs fed with different lysine, protein or energy levels in feed. The 3 trials were performed on weaners between 7 and 10 weeks of age in two different farms.

In trial 1, pigs with higher level of crude protein (CP), net energy and lysine in feed showed the worse average daily gain (ADG). The level of haptoglobin increased significantly in this group of pigs. In trial 2, feeds only differed by their crude protein and lysine levels. Haptoglobin did not show difference between groups. The feed with higher lysine and CP levels had better ADG. In Trial 3, CP levels of 15, 17, 19% in the feed showed no impact on haptoglobin level. ADG showed a negative correlation with haptoglobin at 10 weeks.

Further work is needed to validate if lysine or protein excess could enhanced an acute phase response in pigs at the end of the post-weaning period. Haptoglobin evaluation, as a routine tool, could help to investigate bad growth performances during the post weaning period in farms.

**Keywords:** haptoglobin, piglets, lysine, protein, requirements, growth, average daily gain

### INTRODUCTION

Haptoglobin is one of the major acute phase proteins in pigs (Eckersall 1996). It has been used to identify clinical, subclinical diseases (Sorensen 2006 – Para 2006) and stress situations (Pineiro 2007). For these reasons it has been proposed as a screening parameter in health management systems in piglet rearing. Haptoglobin could help to detect disturbances of homeostasis leading to poor health and bad growth performances (Gymnich 2004). Bad sanitary conditions during the post weaning period lead to an increase of haptoglobin (Le Floch 2006).

During inflammatory states or immune challenges nutrient requirements change (Humphrey 2003 – Obled 2003). The optimal level of amino-acid could be different especially for lysine, essential amino acid for pig growth (Williams 1997). Nutrition is also able to modulate the immune response (Ilsley 2005). The protein metabolism is deeply modified during inflammatory state (Sandberg 2007 – Le Floch 2004). Tryptophan and threonine decrease while lysine increases in piglets submitted to bad sanitary conditions during the post-weaning period (Le Floch 2006). In poultry, it has been demonstrated that deficiency of certain amino acids diminished

many components of the immune response (Takahashi 1997– Konashi 2000). Lysine requirement under immunological stress are decreased, and added lysine does not lead to better growth (Klasing and Barnes 1988, Robert 2005).

The objective of this work is to evaluate in 3 nutritional trials the relationship between feed characteristics, haptoglobin level and growth performances of piglets during the post-weaning period. We focused on routine feed characteristics: the level of lysine and protein, because most of the papers deal with specific nutrients or amino acids. Another purpose was to evaluate the interest of routine haptoglobin measurements in nutritional trials.

## MATERIALS AND METHODS

### Animals and farms

The three trials were performed during the post weaning period (from weaning to 70 and 82 days of age). Trial 1 took place in a commercial farrow to finish farm. The piglets (Landrace X Large White X Pietrain) were weaned at 28 days. Weights were registered by pen. Trials 2 and 3 were conducted at the St. Symphorien research station, in the same post-weaning unit. This post-weaning unit is separated in 48 identical pens of 1.84 m<sup>2</sup> surface. Sows are crossed Landrace and Large White. The boars are from the commercial bread Master, provided by France Hybrid. All piglets were weaned at 21 days. They were weighted individually.

### Haptoglobin analysis

Haptoglobin was quantified in sera. Blood samples were taken from the vena cava. We used a single radial immunodiffusion Plate Test (code P0305 –1 Cardiotech Services Inc, Louisville USA). The agar gel plate contained rabbit antibodies specific for pig haptoglobin. Pig sera were diluted and distributed in the wells of the agar plates. After 24 hours of incubation, a precipitin ring appears. Its diameter is proportional to the concentration of haptoglobin. The test was performed according to the manufacturer except for the reference curve. We used a five points curve – instead of a two points – by diluting the provided standards. The curve requires a  $R^2 > 0.99$  to validate the plate.

### Statistical analysis

GLM Univariate procedure (SPSS 14.0) was applied for all growth data. Experimental groups and sex were fixed effect factors. The mixed model procedure (SPSS 14.0) was used for Haptoglobin data when obtained at different times on the same animals. Correlation between parameters was analysed using Pearson's correlation test.

### Experimental designs

#### Trial 1

96 piglets were distributed according to weaning weight in 8 pens of 12 piglets. Feed was provided *ad libitum*. A fixed amount of prestarter diet was fed before changing to the starter diet (table 1). Half of the piglets received feed with lower level of energy, crude protein and lysine (group 1B). The feeding schedule is shown in table 1. The piglets were followed up, in the post-

weaning unit, from 28 to 70 days of age. Pig weights were recorded by pen at 28, 49 and 82 days. Blood was collected from the same 20 pigs in each feeding group at 48 and 70 days.

**Table 1.** – Trial 1 – Feeding schedule from 28 to 82 days of age (CP crude protein, NE net energy)

	Feed	1A (control)	1B (low level)
	Piglets	48	48
	Pens	4	4
Prestarter diets	Feed 1	2 kg	–
	Feed 2	5 kg	4 kg
Starter diet	NE MJ/kg	9.7	9.5
	CP	18%	17%
	Lysine	1.24	1.05

The pre-starter diets are distributed until the consumption of the specified amount of feed is obtained.

Feed 1: NE 11.5 MJ/kg, CP 20.3, Lys 1.57

Feed 2: NE 10.3 MJ/kg, CP 18.8, Lys 1.33

### Trial 2

240 piglets were distributed according to weaning weight in 48 pens of 5 piglets. They were divided into two groups receiving 2 different feeding schedules (table 2). Feed is provided *ad libitum*. The 2B diet has a lower level of crude protein and lysine. The energy level of the 2 starter diets was the same. The piglets were followed up in the post-weaning unit from 21 to 70 days of age. The bodyweight was recorded per piglet at 21, 28, 42, 56 and 69 days. Blood samples were taken at 67 days of age from the anterior vena cava of the same 15 pigs in each feeding group.

**Table 2.** – Trial 2 – Feeding schedule from 21 to 69 days of age (NE net energy, CP crude protein)

		2A (control)	2B (low level)
	Piglets	120	120
Prestarter diets	Weaning – day 42	Feed 1 (2 kg)	–
		Feed 2	Feed 2
Starter diets	NE MJ/kg	9.7	9.7
	CP	18%	16.5%
	Lysine	1.24	1.05

The feed 1 is distributed until the consumption of the specified amount of feed is obtained.

Feed 1: EN 11.5 MJ/kg, CP 20.3, Lys 1.57

Feed 2: EN 10.3 MJ/kg, CP 18.8, Lys 1.33



**Trial 3**

At 42 days, 240 piglets were distributed according to weaning weight in 4 groups (table 3). Each pen included 5 pigs, except the 4<sup>th</sup> group where one more pig was added to evaluate the impact of density. In each experimental group 24 piglets were randomly selected for blood sampling at 42 and 68 days. The piglets were followed up in the post-weaning unit from 42 to 70 days of age. Bodyweight was recorded per piglet at 42, 56 and 69 days. Blood samples were taken from the same 24 pigs in each group at 42 and 68 days.

**Table 3.** – Trial 3 – Experimental groups and feeds. (NE net energy, CP crude protein)

	3A	3B	3C	3D
Piglets	24	24	24	24
Pigs / pen	5	5	5	6
NE (MJ/kg)	9.8	9.8	9.8	9.8
CP	15%	17%	19%	17%
Lysine	1.18	1.18	1.18	1.18

**RESULTS****Trial 1**

## Growth performance

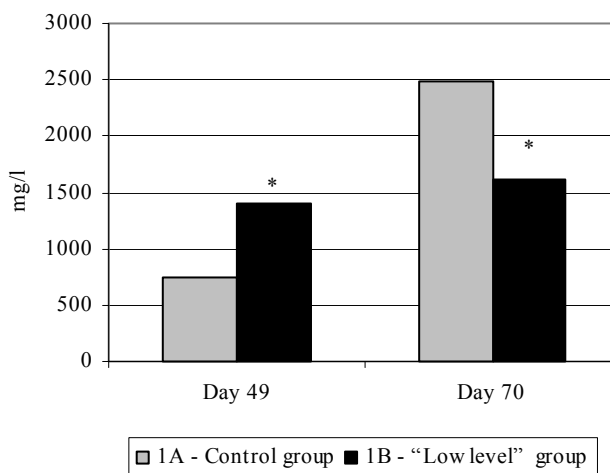
Piglets from the control group showed better average daily gain (ADG) during the pre-starter diets period. The diet with lower levels showed better growth during the distribution of the starter diet. There is no difference in the ADG for the whole post-weaning period. (Table 4)

**Table 4.** – Trial 1 – Average Daily Gain between 28 and 82 days of age – Mean (+/- SD)

	ADG g/d			
	Weight at 28 day	Day 28 to 49	Day 49 to 82	Day 28 to 82
1A – Control group	8.50 (+/-1.25)	424.2 (+/-11.3)	649.2 (+/-27.3)	561.7 (+/-19.9)
1B – “Low level” group	8.39 (+/-1.24)	363.2 (+/-36.5)	720 (+/-44.8)	581.4 (+/-31.8)
<i>F test</i>	<i>P=0.9</i>	<i>P=0.019</i>	<i>P=0.035</i>	<i>P=0.33</i>

## Haptoglobin

The serum haptoglobin level showed a significant increase ( $p < 0.001$ ) from 49 to 70 days of age in the control group (1A). This increase is not observed in the “low level” group. The results between feeding groups differ at both sampling times (figure 1).



**Figure 1.** Trial 1 – Serum Haptoglobin – \* Mean haptoglobin levels differ between groups ( $p < 0.05$ )

## Trial 2

### Growth performance

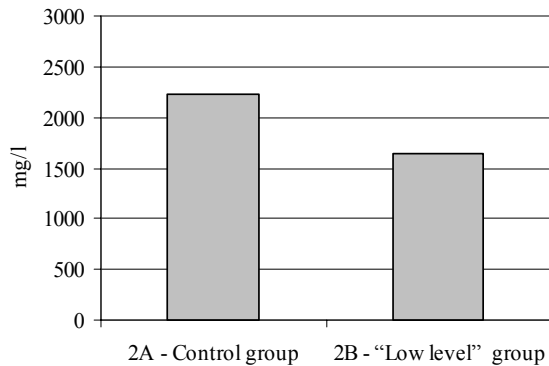
At 21 days the bodyweight was 6.3 (+/-0.9) kg in both groups. The ADG during the prestarter period showed no difference between the groups. The control group had a better growth during the distribution of the starter feed, especially during the first 14 days (table 5).

**Table 5.** – Trial 2 – Average Daily Gain between 21 and 69 days of age – Mean (+/- SD).

Period (days of age)	ADG g/d			
	21–42	42–56	56–69	42–69
2A – Control group	399.0 (+/-41.1)	674.5 (+/-64.9)	824.7 (+/-82.2)	752.5 (+/-43.9)
2B – “Low level” group	410.7 (+/-42.5)	581.7 (+/-83.3)	791.1 (+/-98.7)	690.3 (+/-68.8)
<i>F test</i>	$P=0.45$	$P=0.002$	$P=0.32$	$P=0.006$

### Haptoglobin

At the end of the post weaning period the average level of haptoglobin was respectively 2226 and 1650 mg/l in the control and the “low level” group (figure 2). There is no significant difference.



**Figure 2.** Trial 2 – Serum Haptoglobin at day 67

### Trial 3

Growth performance

The group 3A (low level of crude protein) showed the worse ADG during all the periods (table 6).

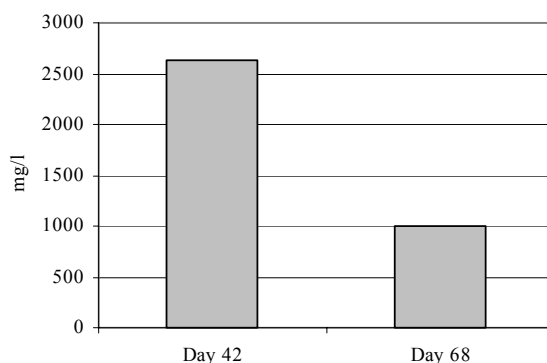
**Table 6.** – Trial 3 – Average Daily Gain between 42 and 69 days of age – Mean (+/- SD)

	N	Day 42 to 56	Day 56 to 69	Day 42 to 69
3A	24	512.8 <sup>a</sup> (+/-110.7)	709.6 <sup>a</sup> (+/-111.3)	614.8 <sup>a</sup> (+/-104.0)
3B	24	609.2 <sup>b</sup> (+/-95.0)	780.2 <sup>bc</sup> (+/-90.9)	697.9 <sup>b</sup> (+/-66.9)
3C	24	590.4 <sup>b</sup> (+/-97.0)	825.2 <sup>c</sup> (+/-86.8)	712.13 <sup>b</sup> (+/-72.0)
3D	23	590.5 <sup>b</sup> (+/-117.9)	765.1 <sup>b</sup> (+/-85.2)	681.0 <sup>b</sup> (+/-95.1)
<i>F test</i>		<i>P</i> =0.006	<i>P</i> =0.001	<i>P</i> =0.001

<sup>abc</sup> Figures in the same column with different letters differ significantly.

### Haptoglobin

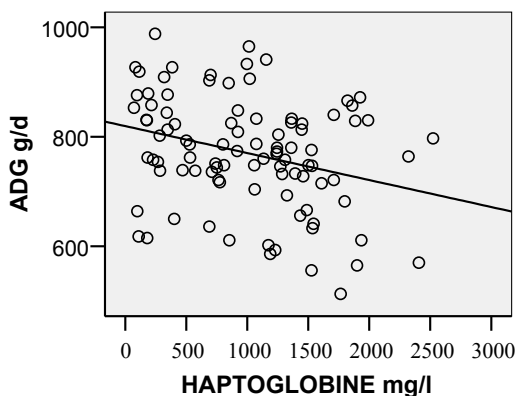
There is no correlation between the individual haptoglobin levels of the pigs of 42 and 69 days of age. The haptoglobin level decreases significantly between 42 and 67 days ( $p < 0.001$ ) (Figure 3). There is no significant difference in haptoglobin levels between groups at 42 and 67 days.



**Figure 3.** Trial 3 – Serum Haptoglobin at day 67

There was a sex effect at 67 days, males showing a level of 896.8 mg/l and females 1146.7 mg/l ( $p=0.049$ ). This effect was not observed at day 42.

The pig density ( $\text{kg/m}^2$ ) in the pen, calculated with the effective pig weights in each pen at 69 days showed no correlation with the haptoglobin level at 42 and at 67 days of age. The pig density at 69 days showed an average of  $86.5 \text{ kg/m}^2$  and ranged from 65 to  $117 \text{ kg/m}^2$ . The ADG from days 56 to 69 and for the whole period showed a significantly negative correlation with the haptoglobin levels at 68 days (fig 1 – table 4).



**Figure 4.** Trial 3 – Relationship between ADG from 56 to 69 days and haptoglobin at 67 days

**Table 7.** Trial 3 – Correlation between haptoglobin and ADG

	ADG 42–56 days	ADG 56–69 days	ADG 42–69 days
Haptoglobin at 42 days	NS	NS	NS
Haptoglobin at 67 days	NS	$R=-0.295$ $P=0.004$	$R=-0.237$ $P=0.021$

## DISCUSSION

Trial 1 leads to unexpected results. There is no difference of ADG between the standard and the “low level” feed (1B). The growth pattern between groups is different. During the first period (weaning to 49 days), the ADG obtained with the standard feed (group 1A) is better than the low level feed. But from day 49 to 82, the opposite situation is observed. During the same period of time, the haptoglobin level increases significantly in group 1A. The average pig weight was 39.3 kg at the end of the post-weaning unit. The lower levels in the starter diet of the 1B group were more suitable for pigs with more than 35 kg bodyweight (Brossard 2007). It does not explain the worse ADG of the 1A group, except if an excess of lysine, protein or net energy has negative impact on growth. Sauerwein 2005 observed the same situation. An increase of the haptoglobin level between 7 and 10 weeks was associated with decreased ADG. In Sauerwein’s trial the increase of haptoglobin was due to an outbreak of respiratory symptoms. We did not observe any specific symptoms in the pigs of the 1A group. The increase of haptoglobin could be attributed to a subclinical inflammation state (Sorensen 2006). It was not possible to measure individual pigs’ weights, because trial 1 was done in a commercial farm. Bodyweight was measured by pen. Only few pens (4 for each experimental group) were used. All the pens were located in the same room. It would be surprising that a subclinical infection affects only the pens of the same experimental group. Nevertheless, it is not possible to strictly conclude that the composition of the diet – higher energy, crude protein and lysine level – is responsible for an enhancement of the acute response. We already observed the same situation in poultry (Robert 2005). A diet with higher level of protein and lysine at the time of vaccination seemed to enhance the inflammatory response of animals.

Trial 2 used the same kind of feeding schedule than trial 1, except the net energy levels which were the same for both starter diets. Unfortunately blood was only sampled at the end of the trial and only from 15 pigs instead of 20 in trial 1. The growth performance of the starter diet (42 to 69 days) was in accordance with our expectation. The 2A group (higher lysine and crude protein level in the starter diet) did not show better ADG after 56 days anymore. That is in accordance with the decrease of lysine needs at the end of the post-weaning period. Haptoglobin levels in the groups are very similar between both trial 1 and 2 at 10 weeks of age (1A, 2480 mg/l; 2A, 2226 mg/L, 1B 1610 mg/l, 2B 1650 mg/l). Nevertheless the haptoglobin difference between groups in trial 2 was not significant.

An inflammatory state between 6 and 10 weeks of age could reduce the needs for lysine and/or protein for growth. This hypothesis is in accordance with Williams 1997. More work have to be done to know if an excess of lysine or protein during this period is able to enhance the acute phase response and could have an additional negative effect on performance of pigs.

The lower level of protein in trial 3 showed the worse ADG. Compared with the other trials the level of haptoglobin at the end of the post-weaning period was low in trial 3. It decreased from 42 to 68 days. Compared with other trials, pigs did not express any acute phase response. The tested situations (different crude protein levels and overcrowding) showed no impact on haptoglobin level. Females showed higher haptoglobin levels. That is in accordance with Clapperton 2005. He found higher haptoglobin level on females at 18 weeks. There was a negative correlation between haptoglobin level at the end of the period and ADG. This negative correlation was highly significant during the two weeks before blood sampling but remains significant for the whole starter diet period (42 to 69 days of age). Haptoglobin at the beginning of this period showed no correlation with ADG. Sauerwein 2005 observed the same. Clapperton

2005 worked with fatteners between 18 and 24 weeks of age. He also showed a negative correlation between ADG and haptoglobin at 24 but not at 18 weeks of age.

## CONCLUSIONS

It was not possible to definitely conclude, from this work, that lysine or protein excess could enhance an acute phase response in pigs. Further work is needed to validate this hypothesis. Nevertheless the obtained results demonstrate that pigs can go through an acute phase response without showing any clinical sign. Utilization of feed by the animal is depending on the inflammatory state. So, it seems necessary to measure acute phase protein during feed trials to better analyze the results. Haptoglobin evaluation, as a routine tool, could help to investigate bad growth performances in farms.

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## METHAPHYLACTIC TREATMENT OF PIGLETS COCCIDIOSES WITH BAYCOX<sup>o</sup> 5%: EFFECT ON WEIGHT GAIN, DIARRHOEA – RELATED MORBIDITY, MORTALITY DURING SUCKLING AND NURSING AND OECONOMIC EFFICIENCE

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**Keywords:** coccidiose, Baycox treatment, healthier pigs, higher benefit

### ABSTRACT

After the oral treatment of all piglets 3–5 days after birth with Baycox<sup>o</sup> 5% we found a better intestine health in the suckling and in the flat deck period. In this study there was reduced diarrhoea during suckling and in the flat deck. With the Baycox<sup>o</sup> therapeuticum the treatment against *E. coli* and *Clostridium perfringens* type C could be decreased for nearly 40% during the breeding period. All pigs got better health status with higher weight gain and uniformity. The Baycox<sup>o</sup> treatment resulted a better monetary benefit during increasing the body weight.

### INTRODUCTION

In the first weeks after birth many piglets have problems with diarrhoea. Endoparasites, enteric bacteria and viral infections have an economic impact on the profitability of pig production through reduction in daily weight gain and in higher losses during fattening. In piggeries diarrhoea of piglets is an important disease during suckling, associated with coccidiose and bacterial infections. Coccidiosis in nursing piglets is a disease caused by *Isoospora suis* and is found in all types of farrowing facilities and under all types of management systems (9). If *I. suis* has established in a farm it is maintained through piglet to piglet transmission, by infected suckling sows and by the contaminated farrowing floor. Reports about the piglets coccidiose caused by *Isoospora suis* demonstrate a high farm prevalence of the disease in different European countries. Overall *I. suis* was found in 26% litters and in 69% farms (12). In the farms in our region with a high pig density we watch, that a coccidiose favoured the increasing of the pathogenicity of enteric diseases in growing pigs. With the study we investigated the relationship between the treatment with Baycox<sup>o</sup> against *I. suis* and the economic efficiency for the pig herds.



## MATERIAL AND METHODS

During the farm visits of the vets they looked for the health status of the pigs by clinical monitoring and collected different samples for laboratory tests. During the visits together with the farmer we controlled the performance. In our region 3–5 days old piglets get an oral metaphylactic treatment with Baycox° against *I. suis*. In many farms with diarrhoea, after a clinical diagnose and a laboratory test by the vets the pigs were treated against *E. coli* or clostridiosis. In this study we collected the different therapeutic and economic dates during fattening.

## RESULTS

In the faecal samples from the litters with diarrhoea we found oocysts of *I. suis*. In the samples from the younger piglets were more oocysts (56%) than in the older once; in the litters with diarrhoea were 86% oocysts (Table 1). In the faecal samples from different farms, tested for bacterial, virus and parasitic colonisation, before the treatment against *I. suis*, we found more gains than after the oral application of Baycox° (Table 2). The weaning weight in the treated group was one kg higher than in the control group. At the end of nursing the body weight in the Baycox° group was 3.5 kg higher (Table 3). In the treated group during the suckling period the pigs showed a diarrhoea reduction to 98% and a mortality reduction to 50% (Table 4). During suckling and nursing in the Baycox° treated group we found a 50% reduction of the mortality (5). The weight uniformity in the different weights groups was in the treated groups higher at the end of the nursing period (6). The Baycox° treatment resulted in monetary benefit because of better weight gain to the tune of 0.93 to 1.33 €.

**Table 1.** Clinical examination of farrows (N=94) and detection of oocysts in faecal samples

Parameter	Detection of oocysts (%)
piglets 7–14 days p.p.	56
piglets 15–28 days p.p.	48
litters with diarrhoea	86
litters without diarrhoea	24

**Table 2.** Results for the incidence of germs in faecal samples (N=281) of piglets (5–21 days p.p.) before and with Baycox° 5% therapy

Probations (N=281)	Without Baycox° treatment (%)	With Baycox° treatment (%)
oocysts	89	6
<i>E. coli</i>	62	24
<i>E. coli</i>	14	11
<i>Cl. Perfringens</i> , type A	81	12
Rotavirus	6	4

**Table 3.** Weight development after metaphylactic Baycox<sup>o</sup> 5% use during 77.2 days

Weight (kg)	Without Baycox <sup>o</sup> treatment	With Baycox <sup>o</sup> treatment
birth weight	1.6	1.6
weaning weight (p=0.0097,significant)	6.0	7.0
end of nursing (p=0.0448,significant)	18.6	22.1

**Table 4.** Baycox<sup>o</sup> 5% effect on diarrhoea and mortality on suckling period

Results (N=391)	Without Baycox <sup>o</sup> treatment (%)	With Baycox <sup>o</sup> treatment (%)
diarrhoea (N=79) (p=0.027,significant)	34.7	0.6
mortality (N=50) (p=0.03,significant)	16.0	8.5

diarrhoea reduction: 98%,mortality reduction: 50%

**Table 5.** Baycox<sup>o</sup> 5% effect on mortality during suckling period and nursery

Results (N=391)	Without Baycox <sup>o</sup> treatment (%)	With Baycox <sup>o</sup> treatment (%)
mortality (N=67) (p=0.029,significant)	12.0	20.9

Mortality reduction during suckling and nursing: 43%

**Table 6.** Baycox<sup>o</sup> 5% effect for uniformity in the weight groups (N=335) at the end of the nursing period

Weight groups (kg)	Without Baycox <sup>o</sup> treatment (%) (N=179)	With Baycox <sup>o</sup> treatment (%) (N=156)
<15	8.4	1.4
15–19	16.2	11.6
19–23	21.2	26.0
23–27	33.5	24.7
27–31	15.1	20.6
31–35	3.4	11.6
> 35	2.2	4.1

## DISCUSSION

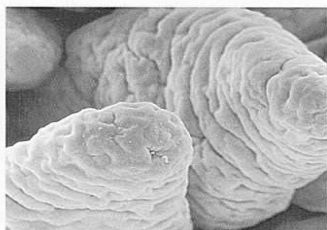
*I. suis* is a cause of diarrhoea on piglet-rearing farms. It is a primary pathogen and the occurrence correlates positively with the occurrence of diarrhoea at the age of 2–3 weeks (6). In the first weeks after birth different germs produce clinical problems with enteritis under the piglets. The reasons for diarrhoea are different germs after birth (Table 7).

**Table 7.** Reasons for enteritis and clinical symptoms during the suckling period

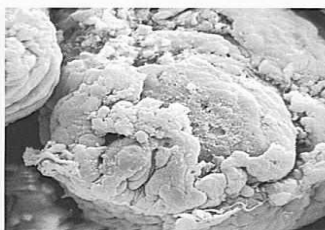
Germ	sickness symptoms
Rotavirus	apathy, vomiting, yellow-pasty enteritis
Coronavirus	vomiting, wasting, slight enteritis
TGE Virus	vomiting, grey-yellow foul smelling enteritis
EVD Virus	grey-yellow enteritis
KSP Virus	fever >41°C, foul-smelling, bloody enteritis
<i>E. coli</i>	aqua-yellow or dilute, brown enteritis
<i>Cl. perfringens</i>	fluid red brown enteritis, with blood
Coccidiosis	yellow pasting, fatty steatorrhoea

In the blood and faecal samples were selected different germs for infections. The oocysts of *I. suis* were found by a flotation medium with NaCl and sugar under UV-light of a fluorescence-mikroscope (2). The “metaphylactic” treatment with Baycox<sup>o</sup> in the piggeries prevented the appearance of clinical signs, better immunity against different germs, a better intestinal morphology allowing the development of immunity. The pathomorphological examinations showed that on average the intestinal villi length was longer on days 10 and 14 in animals treated with Toltrazuril compared with the other groups (13). The link between oocysts excretion and clinical signs seems to be precluded by interaction between parasites, animals, management and the environment (3). In commercial units with grower-finisher pigs in Great Britain they found interactions between the level of dietary fibre and infestation with endoparasites and between infection with *L. intracellularis* and infestation with *Trichuris suis* in grower pigs (8). In the study the incidence of diarrhoea in the Baycox<sup>o</sup> treated group were more reduced than those of the control. In many farms the therapeutic programs could be stopped after the Baycox<sup>o</sup> medication, as the pigs were healthier. In the trial the Baycox<sup>o</sup> treated pigs gained a higher weight. At the time of weaning the Baycox<sup>o</sup> treatment resulted a better weight uniformity. In the study the diarrhoea-related morbidity dropped during the suckling period. The total mortality in the Baycox<sup>o</sup> treated group was more reduced compared with the untreated group.

Intestinal picture (Bayer Animal Health, 2003)



intestinal  
obB



villidestructed intestinal  
5 days post infection



villidestructed intestinal villi  
7 days post infection

In the early Baycox<sup>o</sup> treated piggeries the piglets had a better weight gain, more uniformity, reduced diarrhoea and lower therapeutic costs in the suckling period. In our study we found, that in many farms with the Baycox<sup>o</sup> treatment of piglets the veterinarians and the farmers could reduce the therapy with antibiotics against *E. coli* and haemorrhagic enterotoxaemia. We summarize, by the “metaphylactic” use of Baycox<sup>o</sup> for the piglets, the drugs could be reduced and the pigs had a better health status. In Dutch herds with Toltrazuril treatment the piglet feed intake increased with a better health and on average 789 gram per litter more than the control (5). In a German field study the mean average weight gain on six selected farms at the end of the suckling period was + 376.9g (1). The Baycox<sup>o</sup> treatment resulted in monetary benefits because of better weight.

Disinfection measures did not reduce the maximum incidence of Isosporosis, because the oocysts are very resistant to commonly used disinfectants (4). A *in vitro* study with Neopredisan 135–1 (Menno-Chemie, 22850 Norderstedt) – concentration: 2%, exposure time: 2 hours – reduced 96.96% of the oocysts by lysis (7). In a field study with two farms without *I. suis* therapy and normal disinfection before farrowing the floor was disinfected one and two weeks p.p. again with Neopredisan<sup>o</sup> 135–1 (2%). Afterwards the excretion of oocysts from the piglets was reduced for 43% (11). When the initial contamination of the pen with *I. suis* is high in poorly cleaned pens the majority of the piglets are infected almost soon after birth (10). In a Greece study there was a remarkable variation of the infestation incidences between untreated litters in a *I. suis* contaminated farm. This might be associated to pen related factors such as efficiency of cleaning and disinfection (10).

## CONCLUSIONS

Coccidiosis represents a problem in nursing piglets, especially in countries with an intensive pig production. *I. suis* infected pigs excrete oocysts and infect the whole pen. By the intestinal lesions of the micro villi the piglets are more infected with different germs and have a reduced protection against clinical diseases. The weight gain profile for Baycox<sup>o</sup> groups revealed consistently higher values than the untreated. The piglets have a better weight gain, are healthier without drugs therapy for reducing germs and diarrhoea. Coccidiosis is a disease, which has not only a negative consequence in the piggery but also during the fattening. With a Toltrazuril treatment in the first days p.p. the pig production costs are lower and the benefit is higher till the fattening.

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## STUDY ON THE VIRULENCE AND CROSS-NEUTRALIZATION CAPABILITY OF RECENT PORCINE PARVOVIRUS FIELD ISOLATES AND VACCINE VIRUSES IN EXPERIMENTALLY INFECTED PREGNANT GILTS

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### ABSTRACT

The pathogenicity of two recent German field isolates of *Porcine parvovirus* (PPV-27a and PPV-143a) and two vaccine viruses [PPV-NADL-2 and PPV-IDT (MSV)], which are used for the production of inactivated vaccines, was investigated by inoculation of pregnant sows at day 40 of gestation. Post-infection sera of these sows as well as antiserum prepared in rabbits by immunization with the four above-mentioned PPV isolates and with the virulent strain PPV-Challenge (Engl.) were tested for their homologous and heterologous neutralization activities. All antiserum had high neutralization activities against the vaccine viruses, the PPV-Challenge (Engl.) and PPV-143a, but much lower activity against PPV-27a. These results suggest that PPV-27a represents a new antigenic variant or type of PPV and vaccines based on the established vaccine viruses may not be fully protective against this field isolate. PPV-27a has been characterized based on the amino acid sequences of the capsid protein as a member of a new and distinct PPV cluster. Interestingly, the homologous neutralizing antibody titres of the sera of all three pigs and both rabbits inoculated or immunized with PPV-27a were 100- to 1000-fold lower than the heterologous titres against any of the other viruses. The low homologous neutralizing antibody titres suggest a possible, yet undefined, immune escape mechanism of this PPV isolate.

### INTRODUCTION

*Porcine parvovirus* (PPV) is a member of the family *Parvoviridae*. PPV is widespread in swine herds, despite vaccination. The virulent strains cause reproductive failures in swine, represented by stillbirth, embryonic death, infertility (SMEDI-syndrome) and delayed return to oestrus. The manifestation of clinical disease depends on the pathogenicity of the virus and on the stage of gestation. Fetuses infected before day 70 of gestation usually die, whereas fetuses infected at a later time point develop antibodies against PPV, eliminate the virus and survive the infection.

PPV strains can be distinguished by their different pathogenicity. Substitution of only a few residues in the VP2 capsid protein is thought to be responsible for distinct biological properties.

Phylogenetic analysis of the VP1/VP2 protein gene revealed that there is a relatively weak sequence similarity between PPV-NADL-2 and recent field isolates from Germany.

The aim of the study was to examine two of these recent field isolates, one from each cluster, under experimental conditions for their pathogenicity (*in vivo*) and antigenicity (*in vitro*), particularly in comparison to the vaccine viruses PPV-NADL-2 and PPV-IDT (MSV).

## MATERIAL AND METHODS

**Animal experiment.** Twelve specific-pathogen-free Pietrain x Large White sows, 11 months of age, were randomly assigned to four groups. Groups were kept separately throughout the experiment. At day 40 of gestation the sows were inoculated with the respective viruses by both the intranasal (i.n.) and intramuscular (i.m.) route. Clinical signs (general performance, respiratory activity, food and water intake, and rectal temperature) were recorded daily for 50 days postinoculation. Blood samples were taken in intervals and analysed for antibodies against PPV. At day 90, about three weeks before term, all gilts were euthanized and the fetuses were aseptically delivered via Caesarean and euthanized. Blood and tissue samples were collected from the sows and all fetuses.

**Polyclonal sera.** To prepare virus-specific sera for cross-neutralization tests with the selected field isolates, vaccines viruses and PPV-Challenge (engl.), rabbits were immunized with CsCl-density-purified virus. The resulting sera were heat-inactivated and stored frozen at  $-20^{\circ}\text{C}$ .

**Serology.** Hemagglutination inhibition test for detection of PPV-specific antibodies and serum neutralization test for checking cross-neutralization activity are described in detail in Zeeuw, E. J. et al. (2007).

**Virus detection.** SPEV cells were used for virus reisolation from lung and kidney of the fetuses. Viral DNA was detected by real-time PCR as described by Wilhelm, S. et al. (2006).

## RESULTS

**Clinic.** All sows remained clinically healthy. Fetal mummification was significantly ( $P < 0.05$ ) higher in the gilts infected with PPV-27a as compared to the other groups (85% vs. 5–18%). Almost all fetuses of the gilts of the group 2 infected with PPV-27a showed various ranges of fetal mummification. In contrast, only single mummified fetuses were found in litters of the gilts of groups 1 (PPV-143a), 3 (PPV-IDT [MSV]), or 4 (PPV-NADL-2).

**Serology.** Gilts infected with PPV-143a, PPV-27a and PPV-NADL-2 developed a significant ( $p < 0.05$ ) higher serological response at 2 weeks p.inf. compared to PPV-IDT (MSV). Umbilical cord blood of the non-mummified fetuses from all groups revealed HI antibody titre (Table 2), indicating transplacental infection of all PPV-isolates examined. Neutralizing antibody titres were determined in the post infection sera of the sows and rabbit sera raised against the various PPV-isolates. The neutralizing antibody titre in sera raised against PPV-143a, PPV-IDT (MSV), PPV-NADL-2 and PPV-Challenge (Engl.) against the PPV-Isolate 27a were generally very low, with SN titres ranging from 0.5–0.69, but high against PPV-143a, PPV-IDT (MSV), PPV-NADL-2 and PPV-Challenge (Engl.). Sera raised against PPV-27a neutralized all heterologous PPV-isolates with high titres ranging from 2.99–3.99 (overall geometric mean titre), the homologous virus, however, was less efficiently neutralized (0, 69–1, 19, see table 4).

Virtually identical results were obtained with rabbit sera raised against the PPV isolates, with SN titres of antiserum raised against PPV-27a ranging from 2.29–3.99 against all heterologous viruses, but only titres of 0.69–1.39 against the homologous virus.

**Virus detection.** After two passages, no evidence for virus replication was observed in the fetuses of group 1 (PPV-143a), group 3 (PPV-IDT [MSV]) and group 4 (PPV-NADL-2). In contrast, virus could be readily isolated from fetuses of group 2 (PPV-27a). Viral DNA could be detected by PCR in virtually all mummified and non-mummified fetuses of the PPV-27a inoculated sows, and in single non-mummified piglets of the other groups. However the viral

loads differed dramatically (by a factor of  $10^9$ ) between PPV 27a piglets and those of the other groups.

## DISCUSSION

The fact that in this study antibody and viral DNA could be detected in fetuses of all four groups provides indirect evidence for transplacental infection of both the PPV isolates and the vaccine viruses PPV-IDT (MSV) and PPV-NADL-2. This is in contrast to previous reports where it was postulated that PPV-NADL-2 is not able to cross the placental barrier. But the direct proof for transplacental transmission, the virus reisolation of infectious virus is still missing.

A difference in virulence of PPV-27a to members of the other cluster (PPV-143a, PPV-IDT [MSV], PPV-NADL-2) was indicated by the high mortality of the fetuses. PPV spreads inside the uterus from fetus to fetus. Virus spread was probably more slowly between the fetuses of the groups PPV-143a, PPV-IDT (MSV) and PPV-NADL-2 than between those of the group PPV-27a.

In the present study we investigated in two independent cross-neutralization tests post infection sera of pigs and antiserum of rabbits immunized with the respective viruses. Cross-neutralization of the sera rose against the vaccine viruses PPV-NADL-2 and PPV-IDT [MSV], against the field isolates PPV-143a and PPV-27a as well as against the PPV-Challenge (Engl.) revealed low neutralization activity (0.5–0.69) against PPV-27a, indicating an incomplete protection. Therefore, if PPV-27a is representative for current PPV-isolates in the population, this indicates that vaccines, which are used since 30 years, may no longer be fully protective.

The phylogenetic cluster containing the German isolate PPV-27a is defined by three amino acid substitutions (Q228→E, E419→Q and S436→T) in VP2 (Simpson, A. A. et al., 2002b ; Soares, R. M. et al., 2003 ; Zimmermann, P. et al., 2006 ). All three residues are located in accessible regions on the capsid's surface and position 228 was identified to be part of one of the nine known linear epitopes on VP2 (Kamstrup, S. et al., 1998 ; Simpson, A. A. et al., 2002a ). To what extent the capsid structure will be altered by changing amino acid 228 from Gln to Glu and amino acid 419 from Glu to Gln, and whether they are even involved in the apparent immune escape, needs to be further investigated.

## CONCLUSIONS

In conclusion, our results indicate that possible antigenic variation represented by PPV-27a may influence the effective vaccination against PPV. Further studies and animal inoculation experiments using PPV-27a mutants will be required to address this important issue.

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## CHANGES IN CALPROTECTIN CONCENTRATION IN SOW'S MILK THROUGHOUT LACTATION

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### SUMMARY

Calprotectin is a protein of inflammation from neutrophils and activated macrophages. Faecal calprotectin is a non invasive marker of inflammation of the gastrointestinal tract in humans but little is known on its occurrence in milk. We determined kinetics of calprotectin in colostrums and milk throughout lactation in the porcine species. Milk was collected from fore and hind teats in nine sows between d0 and d29 of lactation (weaning at d28). Calprotectin was found in colostrums and milk of sows. Its concentration varied over time (peak at d5), position of the teat (fore < hind) and sow.

**Key words:** calprotectin, inflammation, lactation, milk, pig

### INTRODUCTION

Calprotectin is a calcium-binding protein of 36.5 kDa that is released at sites of inflammation by neutrophil granulocytes and activated macrophages (Dale et al. 1985). This protein is resistant to degradation and displays anti-microbial properties *in vitro* (Dale et al. 1985; Brandtzaeg et al. 1995). In humans, faecal calprotectin is now considered as a valuable non invasive marker of inflammation of the gastrointestinal tract. Indeed, its concentration is elevated in inflammatory bowel diseases (review by Konikoff et al. 2006). Faecal calprotectin is also positively associated with lifestyle risk factors for colorectal cancer (Poullis et al. 2004). In the porcine species, calprotectin concentration in faeces was shown to be low in piglets at birth, and to increase in the first weeks of life (Lallès and Fagerhol, 2005). It was also 15-fold lower in specific pathogen-free as compared to conventional growing pigs (Lallès and Fagerhol, 2005).

The udder is a site of inflammation, especially around farrowing and after weaning in pigs. However, very little is known on the occurrence and role of calprotectin in this organ. In humans, the presence of calprotectin in milk has been occasionally reported but nothing more is known in connection with the cycle of lactation and on the possible transfer of calprotectin to the young via the milk.

As preliminary assays carried out in our laboratory indicated the presence of calprotectin in porcine milk (J.P. Lallès, unpublished), the aim of the present work was to investigate the changes in calprotectin concentration in colostrums and milk throughout lactation in sows.

## MATERIALS AND METHODS

### *Animals and colostrums and milk sample collection*

Nine pregnant Landrace x Large White sows from the experimental herd of INRA Saint-Gilles, France, were randomly selected for this study. All the sows were in good condition and none of them displayed any clinical sign of diseases. They were in the second to sixth parity and they nursed 11–14 piglets each. Colostrums were collected the day before (d-1) and the day (d0) of farrowing. Milk was collected at various times (d5, 7, 14, 21, 28) during lactation and once after weaning of the progeny (d29). Milk samples (15 to 30 ml) were obtained from two fore teats and two hind teats in the mornings (between 9.00 and 11.00) a few minutes after oxytocin administration intravenously in the ear (1.5 ml of oxytocin at 10 IU/ml). The two samples at each teat location were immediately pooled. The samples were frozen at  $-20^{\circ}\text{C}$  until all the samples of the kinetics had been obtained. Then, the milk samples were thawed overnight at  $+4^{\circ}\text{C}$ . They were centrifuged at 4000 rpm for 15 min and the defatted colostrums or milk was collected using a glass Pasteur pipette.

### *Laboratory analyses*

Calprotectin was extracted from defatted colostrums and milk in the extraction buffer (1:1, vol: vol; Calprest, Eurospital, Trieste, Italy) as used for faeces (Lallès and Fagerhol, 2005).

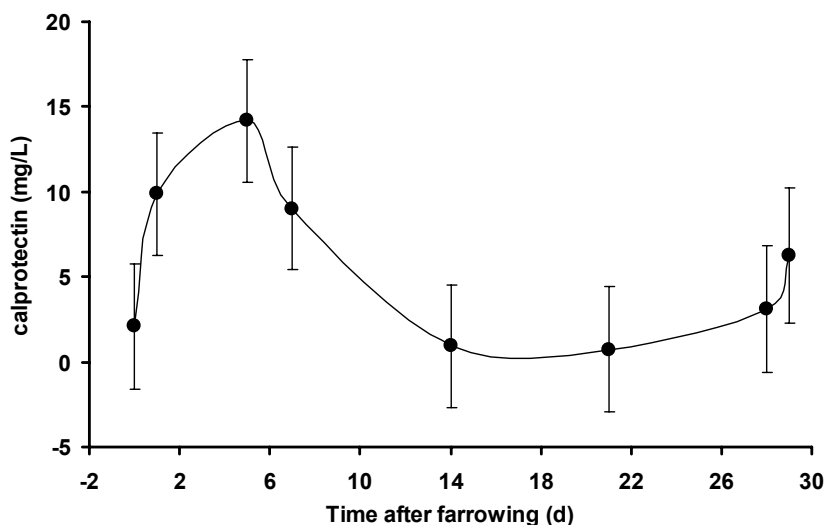
Calprotectin was assayed in these samples using a sandwich ELISA (Lallès and Fagerhol, 2005) following a protocol similar to that published for human calprotectin (Ton et al. 2000). In parallel, total protein was also determined in the defatted samples colorimetrically according to the procedure of Lowry et al. (1951).

### *Statistical analysis*

Calprotectin data, contrary to total protein data were not distributed normally so they were  $\log_{10}$ -transformed before statistical treatment. The experimental unit was the sow. Data were analysed using the PROX MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA) for testing the effects of time, teat position and sow and interaction between time and teat position. Significant effects were declared at  $P < 0.05$ . Data are presented as least-square means and SEM.

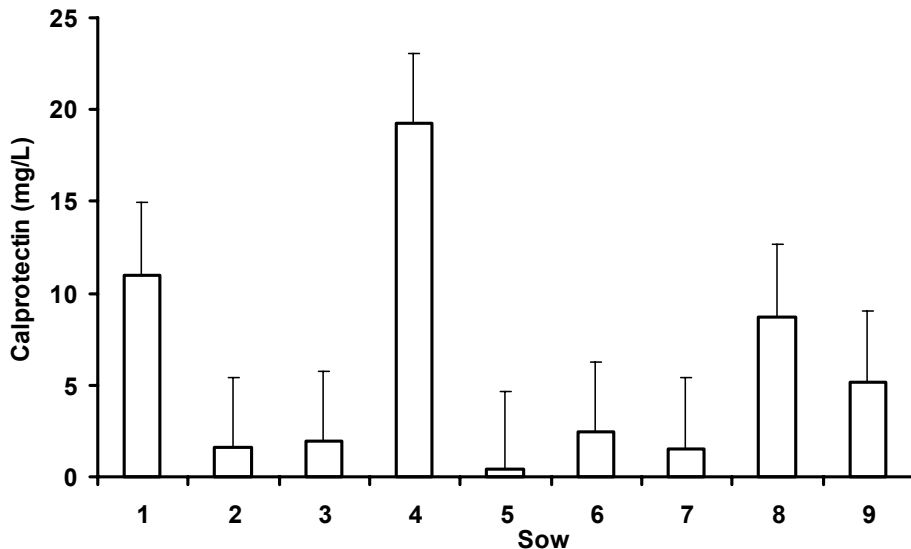
## RESULTS

Total protein concentration over time in defatted colostrums and milk was influenced by the time ( $P < 0.0001$ ), teat position ( $P < 0.05$ ) and sow ( $P < 0.05$ ) with no significant interaction between the time and teat position ( $P > 0.05$ ). Total protein concentration decreased from  $179 \pm 6.4$  g/L in colostrums the day of farrowing to  $56 \pm 6.4$  g/L in the milk collected on d5 after farrowing without significant changes thereafter (mean concentration of  $52 \pm 2.5$  between d7 and d29). Total protein concentration was 16% higher in the hind teats as compared to the front teats ( $79 \pm 3.2$  and  $68 \pm 3.3$  g/L, respectively).



**Figure 1.** Changes in calprotectin concentration in defatted colostrums and milk of sows ( $n = 9$ ) throughout lactation (weaning at d28) (Ismeans  $\pm$  SEM).

Calprotectin concentration in defatted colostrums and milk was very variable. It was influenced by the time, the teat position and the sow ( $P < 0.0001$ ), the time by teat position interaction being non-significant ( $P > 0.05$ ). Calprotectin concentration increased during the first days of lactation and then decreased progressively (Figure 1). Calprotectin concentration was 3.5-fold higher in the hind teats as compared to the fore teats ( $9.0 \pm 1.8$  and  $2.6 \pm 1.9$  mg/L, respectively). This difference was more marked during the first week of lactation. Calprotectin concentration was influenced by the sow, two sows displaying values higher than 10 mg/L, two sows having values comprised between 5 and 10 mg/L and five sows showing much lower values ( $< 2.5$  mg/L) (Figure 2).



**Figure 2.** Influence of the sow on calprotectin concentration in defatted colostrums and milk (lsmeans  $\pm$  SEM).

## DISCUSSION

It is known that oxytocin alters protein composition of milk in cows (Alex et al. 1985). Here we administered systematically low doses of oxytocin (15 IU per sow) as compared to higher doses (40 IU per sow) used in another study in sows (Elliot et al. 1984). This may have minimised the possible effect of oxytocin on protein composition of sow's colostrums and milk in the present work.

Total protein concentration and its observed decrease between colostrums and milk at the beginning of lactation are in agreement with previous data in the porcine species (review by Gallagher et al. 1997). However, this review emphasized that such a decrease does not occur uniformly for all protein types. This finding is supported further by the lack of correlation between total protein and calprotectin here.

The present results clearly show that calprotectin was present in colostrums and milk of sows throughout lactation. A low concentration of calprotectin was observed at farrowing suggesting a low level of mammary gland inflammation at that time. This observation is also in agreement with the low faecal calprotectin levels found in baby pigs at birth (Lallès and Fagerhol, 2005). The following increase in milk calprotectin level suggests an enhanced mammary gland inflammation during the first days of lactation. Whether it is stimulated by the process of suckling itself is presently unknown. As faecal calprotectin also increased during the first days of life in piglets, one question then arises on the origin – maternal via the milk or endogenous – of faecal calprotectin in piglets.

The kinetics of calprotectin concentration in colostrums and milk as revealed here appeared to differ substantially from that of lactoferrin. Lactoferrin is a multifunctional protein with anti-inflammatory and bacteriostatic properties, found in milk, and originating from glandular

epithelial cells and neutrophil granulocytes (review by Ward et al. 2005). Lactoferrin concentration was found to be high in sow's milk during the first week of lactation in two studies (Elliot et al. 1984; Yang et al. 2000). Ceruloplasmin, an acute-phase protein enhanced by inflammation and inflammatory cytokines is also higher in early than in late lactation in sows (Cerveza et al. 2000). Despite distinct kinetics, all these proteins display higher levels in early lactation, indicating this period is critical for inflammation.

Another interesting point of this study is the influence of teat position on calprotectin concentrations. The hind teats displayed much higher calprotectin levels than the fore teats, especially during the first week of lactation. One hypothesis is that hind teats would be more exposed to bacterial contamination by sow's faeces. It is known that improving hygiene of the farrowing crate by removing faeces decreased bacterial contamination of the mammary gland and mastitis in sows (Bertschiner et al. 1990). However, to the best of our knowledge no published information is available on a possible link between teat position and inflammation.

## CONCLUSIONS AND IMPLICATIONS

In conclusion, calprotectin was found in the colostrums and milk of sows. Its concentration varied over lactation independently from total protein concentration. Calprotectin concentration in milk depended on the position of the teat, hind teats displaying much higher levels than fore teats. Calprotectin may be a valuable biomarker of mammary gland and teat inflammation. Calprotectin in colostrums and milk might be protective not only to sow's udder but also to the gastrointestinal tract of the progeny.

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## IMPROVING AIR QUALITY IN PIGGERY BUILDINGS

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### SUMMARY

The negative effects of high concentration of bioaerosol on animal health, welfare and productivity are well documented. Reducing the concentration of airborne particles in piggery buildings is therefore an important task and could also help to reduce the occupational health and safety risk associated with farm workers. The objective of this research was to evaluate the effects of spraying a mixture of oil and water directly onto pen floors on the concentration of airborne particles inside piggery buildings. Air quality parameters were recorded in a number of partially slatted, mechanically or naturally ventilated pig facilities. The floor of one of the experimental rooms was sprayed daily with a mixture of canola oil and water (50:50) at the rate of 3 g/pig, using an automatic spraying system, while the other room was not treated (control facility). Airborne pollutant concentrations were measured and compared between the two treatments. The concentration of both inhalable and respirable airborne particles was significantly reduced in the experiment facilities.

**Key words:** pigs, air quality, spraying, reduction, emission, dust, airborne particles

### INTRODUCTION

Dust is one of the major airborne pollutants associated with intensive livestock production and determines the quality of the environment within livestock buildings (Wathes 1994). The negative effects of high concentration of bioaerosol on human and animal health, as well as on animal welfare and productivity are well documented (Donham 1991; Donham & Leininger 1984; Donham *et al.* 1984). Suspended airborne particles can also absorb toxic and noxious gases as well as bacteria components and act as vectors for these pollutants (Donaldson 1977). High concentrations of airborne particles may contain bacterial toxins and appears to enhance both the prevalence and severity of respiratory diseases in pigs (Cargill *et al.* 1998). Reducing the concentration of airborne particles in piggery buildings is therefore an important component of good management and can improve production efficiency and reduce the potentially harmful effects of long term exposure to humans (Donham *et al.* 1989).

In addition, Australian data suggests that an average enterprise of 200–400 sows on a single site would release significant amounts of dust, bacteria, ammonia and endotoxins into the surrounding environment via emissions from buildings (Banhazi *et al.* 2006). Emissions, especially ammonia emissions from pig farms are now very closely regulated in the EU and excessive emissions could result in reduced market access of individual piggery operators (Arogo *et al.* 2001; Phillips *et al.* 2001; Wathes *et al.* 1998). Therefore, simple, low-cost and practical techniques, which will have the potential to deliver a significant reduction of odour, ammonia and other pollutant emissions cost effectively, need to be investigated, developed and evaluated



(Aarnink *et al.* 1997). Spraying the floor of pig sheds with a mixture of oil and water (Takai *et al.* 1995) is a potentially beneficial technique. Furthermore, publications from the USA also indicate that odour and possibly ammonia emissions can be reduced by oil spraying (Jacobson *et al.* 1998).

Therefore, the objective of this project was to evaluate the effects of oil spraying and other airborne pollution reduction techniques, primarily on the concentration of airborne particles inside piggery facilities, but the effects of applications on the concentration of other airborne pollutants were also investigated. As a result of reduced internal concentration levels, marked reduction can also be achieved in pollutant emission, assuming the same level of ventilation.

## MATERIAL AND METHODS

An automated oil spraying system was installed in a number of piggery buildings. Information on the general design concept of oil spraying systems have been published previously (Banhazi 2005; Lemay *et al.* 1999; Takai & Pedersen 1999). Air quality parameters were recorded for 32 days in two partially-slatted, mechanically ventilated weaner rooms housing 89 pigs (approximate mean live weight 18 kg) and for 16 days in two partially-slatted, naturally ventilated grower rooms housing 91 pigs (approximate mean live weight 42 kg). The floor of one of the rooms (experimental facility) was sprayed daily with a canola oil, water and surfactant mixture at a 4:5:1 ratio and at the rate of 3 g/pig ( $6.3\text{g/m}^2$ ), using an automatic spraying system. The other room was not treated and served as a control facility. Air quality parameters (as described below) as well as the growth rate of animals were also measured throughout the trials.

Temperature and humidity data were recorded in all sheds monitored using Tinytalk temperature and humidity loggers (Hasting Dataloggers, Tinytalk-2). The sensors were placed as close to pig level as practically possible, without allowing the pigs to interfere with the instruments. Most loggers were attached to the ceiling or a beam, using wire cable and were lowered to pig level above a selected pen, representing the average condition of the shed. Total inhalable and respirable particle concentrations were measured using air pumps connected to cyclone filter heads (for respirable particles) and Seven Hole Sampler (SHS) filter heads (for inhalable dust) and operated at 1.9 and 2.0 l/min flow rate, respectively. The pumps were operated over a 6 or 8-hour period. The selection of the monitoring period was based on previous studies (Pedersen 1993). After sampling, the filter heads were taken back to the laboratory and weighed to the nearest 0.001 milligram using certified microbalances and then the inhalable and respirable dust levels were calculated.



**Figure 1.** Measurement equipment used during the study included the (1) OSIRIS particle monitoring equipment, (2) Anderson bacteria sampler and the (3) Multi Gas Monitoring Machine, developed in-house.

Continuous dust (OSIRIS-2014, Turnkey Ltd.) monitoring equipment was used in some sheds to collect dust distribution information (Figure 1). Ammonia and carbon dioxide were monitored continuously using a gas monitoring machine (Banhazi *et al.* 2005). The equipment was calibrated (using standard 50 ppm ammonia and 2,500 ppm carbon dioxide calibration gases) as required (Figure 1). Total viable airborne bacteria were measured using an Anderson viable six-stage bacterial impactor (Clarke & Madelin 1987) filled with horse blood agar plates (HBA). The airspace was sampled for five minutes at a flow rate of 1.9 litre/minute (Figure 1). The bacteria plates were incubated for 48 hours at 37 °C and the number of colony forming units was counted manually. The figures were entered in a database and the concentration of airborne microorganisms was calculated and expressed as Colony forming Units (CFUs)/m<sup>3</sup>. The data were analysed using ANOVA procedures (Statistica 6.1).

## RESULTS AND DISCUSSION

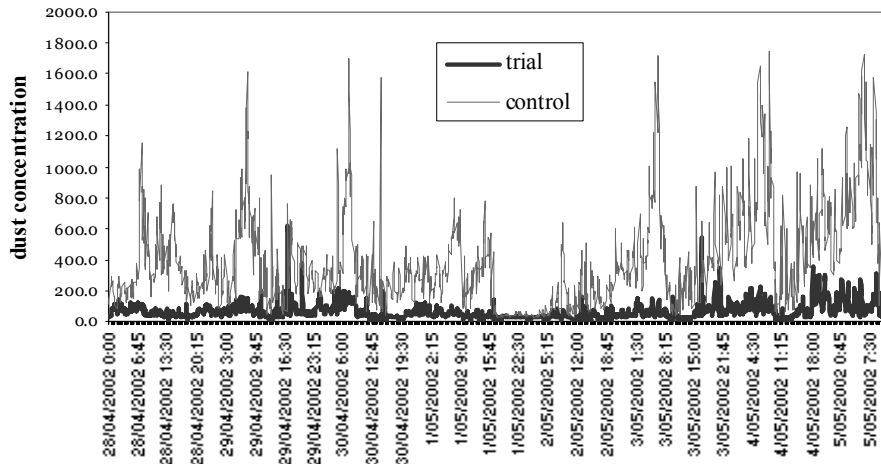
The concentration of both inhalable and respirable airborne particles as well as airborne bacteria was significantly reduced in the experiment facilities (Table 1 and 2).

**Table 1.** Concentrations of respirable and inhalable airborne particles, viable bacteria and ammonia for the control and treatment rooms.

Treatment	Respirable particles (mg/m <sup>3</sup> )	Inhalable particles (mg/m <sup>3</sup> )	Total Bacteria (X 1000 CFUs/m <sup>3</sup> )	Ammonia (ppm)
Weaner (Control)	0.208 <sup>a</sup>	4.023 <sup>a</sup>	67 <sup>a</sup>	11.2 <sup>a</sup>
Weaner (Treatment)	0.150 <sup>b</sup>	2.278 <sup>b</sup>	39 <sup>b</sup>	10.0 <sup>a</sup>
Grower (Control)	0.128 <sup>a</sup>	1.463 <sup>a</sup>	66 <sup>a</sup>	8.7 <sup>a</sup>
Grower (Treatment)	0.106 <sup>a</sup>	0.790 <sup>b</sup>	112 <sup>b</sup>	9.0 <sup>a</sup>

<sup>ab</sup> Values in the same column with different superscripts differ significantly (P<0.05).

Measurement conducted using the OSIRIS optical particle counter demonstrated the visible dust reduction achieved in the experimental facilities. Although the results provided by the OSIRIS equipment were not always reliable in terms of absolute concentrations; these readings demonstrated the relative dust reduction achieved when dust concentrations in the control and experimental facilities were compared in real time.



**Figure 2.** Airborne particle reduction achieved (Osiris measurements) at a piggery during the commercial trials (Sample data shown).

Despite the significant environmental improvement achieved, no significant difference was detected between the growth rate of the treatment and experimental groups. This is in agreement with previously published data (Takai *et al.* 1995).

The experiment achieved its aim of demonstrating a reduction in the concentrations of both inhalable and respirable airborne particles in the airspace following the direct spraying of an oil and water mixture onto the floor. This study confirmed previously published data (Takai *et al.* 1995) and the technique used in the experiment could be used by producers to effectively reduce dust levels in piggery building. However, further studies are needed to determine the long-term effects of frequent oil spraying on subsequent surface hygiene of pen floors. Overall, the technique is a safe and efficient dust reduction method and should be promoted to producers experiencing dust problems in their facilities.

#### ACKNOWLEDGEMENT

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## **THE EFFECT OF A NEW STYLE TRAINING PROGRAM FOR FARMERS ON PIG HEALTH AND PERFORMANCE**

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### **SUMMARY**

To achieve more consciousness at pig farmers about their influence on disease prevention and the immune response of their pigs, farmers took part in a new style training program. Pig farmers indicated that their consciousness about their influence on disease prevention and the immune response was improved. The farmers who participated in the training program undertook significantly more improvements than farmers from the control group. The majority of the trained farmers indicated that the improvements were effective. On average the pigs of the trained farmers had a significant better gut health compared to the pigs of the control group.

**Keywords:** knowledge transfer, management, physiological parameters, pig health, farm performance

### **OBJECTIVE**

The immune response of pigs is complex. Literature shows that management measures taken by the farmer may improve the immune response and therefore prevent diseases (Boersma et al., 2005). A training program should give farmers more insight in the immune response of the pig and in disease prevention. But knowledge transfer is just not enough, newly gained knowledge should also be taken over by the farmers. We tested the effect of a new style training program on a physiological parameter for the immune response, on a parameter for gut health, on pig performance and on the number of improvements taken by the farmer.

### **METHODS AND MATERIAL**

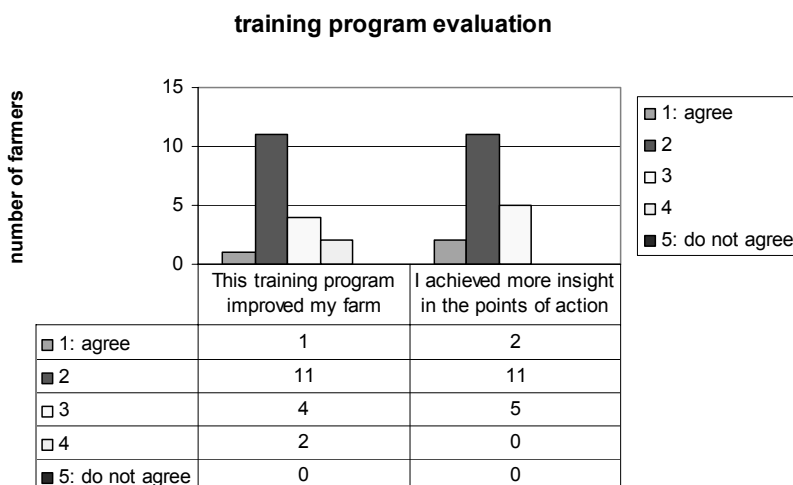
In 2005 and 2006, 35 Dutch pig farmers with multiplication and fattening units took part in the project. To achieve an effect of a training program, knowledge transfer is just not enough. Newly gained knowledge should be taken over by the farmers, to achieve a real effect. Therefore 18 farmers (test group) took part in three meetings. The first meeting consisted of knowledge transfer and farmers discussing their own farms with other farmers at seven themes to achieve more insight in the factors to be optimized at their own farm. Hereby it became evident for the farmers what to improve. The second meeting farmers were focused to achieve insight in ways to improve their farm by discussing their problem together with colleague farmers, veterinarians and researchers on animal husbandry. At the third meeting a plan of action was made with set deadlines. Farmers had to carry out the improvements from the plan of action for a least half a

year. The meetings took place during the months September until December 2005. 18 farmers belonging to the control group did not take part in the training program. The farmers were randomly assigned to the test group or the control group.

Two physiological parameters for pig health were measured both at the beginning and at the end of the trial period of one year: percentage of lymphocytes and I-FABP (Intestinal Fatty Acid Binding Protein). The percentage of lymphocytes indicates the disease resistance in general or the state of health at a certain moment. I-FABP can be measured in blood when leakage of the intestine is present for example due to stress or changes in feed (Niewold et al., 2004). For these parameters blood samples were taken from 30 fattening pigs of 50 kg on each farm. The difference in increase or decrease of both parameters between the test and control group was analyzed with a linear regression model (Genstat8, 2005). The tests were performed excluding farms on which the breeding strategy was changed. Farm performance, an evaluation of the training program and the number of improvements taken by the farmer were achieved by means of a questionnaire. Farm performance was measured every three months as average daily gain (ADG) and as feed conversion ratio (FC). A regression model was used to test significance of the differences in the development of these two parameters. The difference on the number of improvements taken by the farmers during the trial period was tested with a generalized linear regression model.

### RESULTS

The farmers from the test group indicated that they achieved more insight in the points of action to improve the immune response of their pigs and prevent diseases as a result of the meetings (Figure 1).



**Figure 1.** Farm evaluation

No significant difference has been found between the two groups for the percentage of plasma lymphocytes (table 1). Maybe the number of viruses and bacteria at the farms did not change within trial period, yet.

**Table 1.** Mean percentage of lymphocytes (excluding farms with change in breeding strategy)

	Test Group (n=9)	Control group (n= 13)
2005	61.55	60.87
2006	62.02	61.73
Change during 1 year	+0.47	+0.86

The average I-FABP level of the pigs on the test farms was lower for farms in the test group compared to farms in the control group. This indicates that on average the pigs from farms in the test group have a better gut health (table 2).

**Table 2.** Median of the category I-FABP>40 (excl. farms with change in breeding strategy)

	Test group (n=9)	Control group (n=13)
2005	110%	100%
2006	139%	173%
Development during 1 year	+28% (*)	+73%

\*: significance: P-value<0.10 (P=0.055)

Note: When analyzed with the 75%-quartiel of I-FABP : P-values<0.05

ADG and FC were significant better for the test group during the first three months after the last meeting (P< 0.001). However, the difference between the two groups diminished 6 months after the last meeting (table 3). The difference between the two groups during the first months after the meetings might be a result of intense attention on the subject. A few months after the meetings it might be that the attention of the farmer diminished and therefore the difference diminished.

**Table 3.** ADG and FC for the test and control group during four periods of three months

Period	ADG (n=17)		FC (n=17)	
	Control (n=8)	Test (n=9)	Control (n=8)	Test (n=9)
Month 9–12 2005	0	0	0	0
Month 1–3 2006	0	30.2(*)	0	-0.24(*)
Month 4–6 2006	0	14.9	0	-0.18
Month 7–9 2006	0	0	0	0.06
Significance (Treatment x Period)	P= 0.18		P<0.001	

\* : significance: P-value<0.05

The average number of improvements was significantly higher at farms of the test group. The training program led to a better consciousness to the different themes on disease prevention and the immune response of their pigs. Table 4 shows the number of improvements per theme.

**Table 4.** Average number of improvements per farm per theme

Theme	Control Group (n=17)	Test Group (n=16)
Average number of improvements per farm	7.4	16.4*
Improvements per theme		
Others (farm size, breed etc.)	0.3	0.5 n.s.
Pig management	1.7	4.1*
Feed and water	1.4	3.6*
Climate	1.1	2.1 n.s.
Pathogen burden/ hygiene/ vaccination	2.2	4.4*
Care of sow and piglet	0.8	1.1 n.s.

\* : significance:  $P < 0.001$ ; n.s.= not significant ( $p > 0.05$ )

### CONCLUSION

This new style training program for farmers raised more consciousness about their influence on the immune response and disease prevention. Therefore farmers applied more improvements on their farms which resulted in a better gut health. However the effects of the meetings on ADG and on FC were only seen a few months after the meetings.

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## POSTER PRESENTATIONS

### THE IMPACT OF MICROCLIMATE FACTORS TO PIGS' HEALTH AND PERFORMANCE

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#### SUMMARY

The scope of this work is to study how the environment can influence the pigs' health and performance.

The study was realized in a pig-farm from Bumbesti-Jiu locality, Gorj County, Romania. The parameters as the concentration of CO<sub>2</sub>, NH<sub>3</sub> and H<sub>2</sub>S and intensity of noise were determined in the following compartments: nursery, young pigs, fattening, using gas-analyzer and sound meter. The determination were made in two moments: first – when in compartment there was only natural ventilation, the second – after introducing of automatic ventilation. In parallel with environmental parameters, there were analyzed the health parameters (morbidity and mortality) and the performance parameters (daily average weight increase and fodder consumption per weight increase).

After introducing of automatic ventilation, it was observed the followings:

- improving of concentration of CO<sub>2</sub>, NH<sub>3</sub> and H<sub>2</sub>S;
- decreasing of morbidity with 20–30%;
- decreasing of mortality with 6%;
- increasing of daily average weight increase with 100g per day for young pigs and 130 g per day for fat pigs;
- decreasing of fodder consumption per weight increase with 0,2–0,3 units for young pigs and 0,9 units for fat pigs

**Keywords:** performance, health, environmental, animal welfare

#### INTRODUCTION

In Romania, the same as all over the world, there is a major concern of the breeders, together with the veterinarians, to ensure the welfare of the animals. One of the acknowledged criteria by which the animal welfare is ensured is also the creation of a comfortable area for the animal, together with its physical security (the absence of discomfort). An essential requirement for the animals' health is to provide the microclimate factors in normal parameters – this means to eliminate the ecological stress non-animated factors (high concentrations of CO<sub>2</sub>, NH<sub>3</sub>, H<sub>2</sub>S, noise).

As a short term strategy, the pig breeders decided to evaluate the state of welfare by indirect methods regarding the “*interrogation*” of the animals, the studies being also required by the

practical farm conditions where the unjustified decrease of productive performance, increased morbidity and mortality are observed. Following the epidemiological investigations, it was established that the determination of the microclimate factors would be one of the priorities.

### Material and methods

A team of veterinarians and assistants went in the morning in Gorj county at the pig farm Bumbesti Jiu, before starting the daily activities (airing by opening the windows, cleaning, feeding...etc.). The concentrations of the gas emissions and the intensity of the sounds were determined with the help of the gas analyzer and sound meter, (CO<sub>2</sub>, NH<sub>3</sub>, H<sub>2</sub>S), in various points from the shelter at various heights, especially in the compartments in which the morbid processes were more dramatic, namely in the maternity, youth and fattening room.

The determinations have been performed in two different conditions:

- the first, in conditions of natural ventilation;
- the second, after a month, in conditions of automated ventilation

The health parameters (morbidity and mortality) and the performance parameters (the average gain in weight and the specific consumption for kilogram of weight) have been analyzed in parallel.

Also, together with these determinations necropsy exams were conducted on the animals found dead.

## RESULTS AND DISCUSSIONS

**Table 1.** Values of the microclimate factors in conditions of natural ventilation

No.	Name of objective	Specification	CO <sub>2</sub> (%)	H <sub>2</sub> S (ppm)	NH <sub>3</sub> (ppm)	Noise intensity (dB)
1	Shed 3 Compartment 2	Entrance to shelter	0,3	7	6	50,24
	Maternity	At animal level	0,3	8	8	–
2	Shed 4 Compartment 3	Entrance to shelter	<b>0,5</b>	6	6	70,2
	Maternity	At animal level	<b>0,5</b>	7	10	–
3	Shed 6 Compartment 1	Entrance to shelter	0,3	5	3	73
	Hatchery phase I	At animal level	<b>0,4</b>	9	2	–
4	Shed 5 Compartment 1	Entrance to shelter	0,3	4	8	77,4
	Maternity	At animal level	0,3	7	11	–
5	Shed 7 Compartment 3	Entrance to shelter	0,3	6	20	58,5
	Youth	At animal level	<b>0,4</b>	<b>15</b>	<b>30</b>	–
6	Shed 8 Compartment 3	Entrance to shelter	0,3	4	5	58,2
	Youth second phase	At animal level	0,3	7	7	–
7	Shed 14 Compartment 1	Entrance to shelter	0	6	7	53,8
	Youth 90 days	At animal level	0	8	7	–

**Table 1.** Continuation

No.	Name of objective	Specification	CO <sub>2</sub> (%)	H <sub>2</sub> S (ppm)	NH <sub>3</sub> (ppm)	Noise intensity (dB)
8	Shed 11 Compartment 4	Entrance to shelter	0	17	27	75,3
	Youth 150 days	In the middle of the shelter	0	24	31	–
		At animal level	0,2	33	44	–
9	Shed 11 Compartment 3	Entrance to shelter	0,2	45	53	64,6
	Youth 130 days	Middle of the shelter	0,2	37	49	70,3
10	Shed 15 Compartment 1	Entrance to shelter	0	8	17	–
	Fattening room	Middle of the shelter	0	9	21	75,24
		At animal level	0	7	20	–
11	Shed 8 Compartment 1	Entrance to shelter	0	5	0	55,4
	Gestation	At animal level	0	9	0	–

The maximum allowed gas concentrations and noise intensity inside the shelter which are stipulated by Romanian veterinarian-sanitarian law, for all the porcine categories raised in the intensive system are as following:

- 0, 3% (3000 ppm) for carbon dioxide (CO<sub>2</sub>);
- 26 ppm for ammonia (NH<sub>3</sub>);
- 10 ppm for hydrogen sulphide (H<sub>2</sub>S);
- 80–90 dB for noise intensity

According to the data analysis, the maximum allowed concentrations have been surpassed for ammonia, carbon dioxide and hydrogen sulphide in the compartments marked in the table; the noise intensity was registered in normal limits.

It is known from the specific literature that all the three investigated gases are involved in the outcome of the morbid processes, becoming toxic for the nervous system, breathing system, blood and mucous membranes when they are in high quantities.

The result of the combination with the cellular enzymes is the cellular anoxia, manifested through a state of agitation, convulsions, cyanosis, digestive upset, all these cumulated leading to an increase in morbidity and mortality percentage which, at that moment, rose to 30–40% and 14% respectively.

After the anatomy pathologic examination, the following table distinguished:

- oedema and pulmonary haemorrhage;
- hepatic – renal congestion,
- fluid and light coloured blood (raspberry colour);
- degenerative lesions of the nerves;
- brain and meninx haemorrhage;
- hepatomegaly in 1% of the examined cases

As a result of this action, the farm's management decided to improve the microclimate conditions by installing automated, high-performance ventilators, specific for the porcine breeding sector.

The protocol that determines the microclimate parameters has been repeated a month after these ventilators had been installed, noting primarily an improvement of the productive performances of the animals, expressed by:

- improvement of the specific consumption from:

- 2.4–2.5 kg forage/kg gain to 2.2 kg forage/kg gain in the youth category;
  - 3.6 kg forage/kg gain to 2.7 kg forage/kg gain in the fat porcine category
- increase in the average weight gain:
- from 400 g/day to 500 g/day in the youth category;
  - from 670 g/day to 800 g/day in the fat porcine category

The morbidity and mortality percentage have decreased to 10% and 8% respectively. The repetition of the determinations regarding the three target noxious gases proved that all the obtained values were below the normal limits allowed by the legislation in force.

## CONCLUSIONS

- the welfare of the farm animals has been a major subject of concern for the breeders and for the general public for a few years;
- there is a also necessity to develop some measurement systems for the welfare of the farm animals, on a scientific basis, to answer among others to the certification demands coming from the farms;
- the selection of the measures for an evaluation system must be based on validity, repeatability and feasibility;
- the improvement of the ventilation system brought an improvement in the health and performance parameters of the animals;
- the evaluation of the animal welfare must be performed on zoo cultural, behavioral and physiological criteria, keeping in mind that the optimum environment for the animal should ultimately answer to the productivity criteria, which will ensure an acceptable way of life for the breeder.

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## AN EXPLORATORY SURVEY OF LUNG LESIONS IN CULLED SOWS

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### SUMMARY

The aim of this small-scale survey was to assess lung lesions in a sample of culled sows in order to prepare further investigations on respiratory diseases in farrow-to-finish pig operations. Lungs of 60 sows belonging to 36 pig herds were collected at slaughterhouse in Brittany. Pneumonia and pleuritis were scored. Healing pneumonic lesions were also recorded. The results indicate that lung lesions are of rather mild severity and that they are found at a low frequency in culled sows. These preliminary findings are in contrast with the high prevalence of pneumonia commonly detected at the slaughterhouse in fattening pigs.

**Keywords:** lung lesions, culled sows

### INTRODUCTION

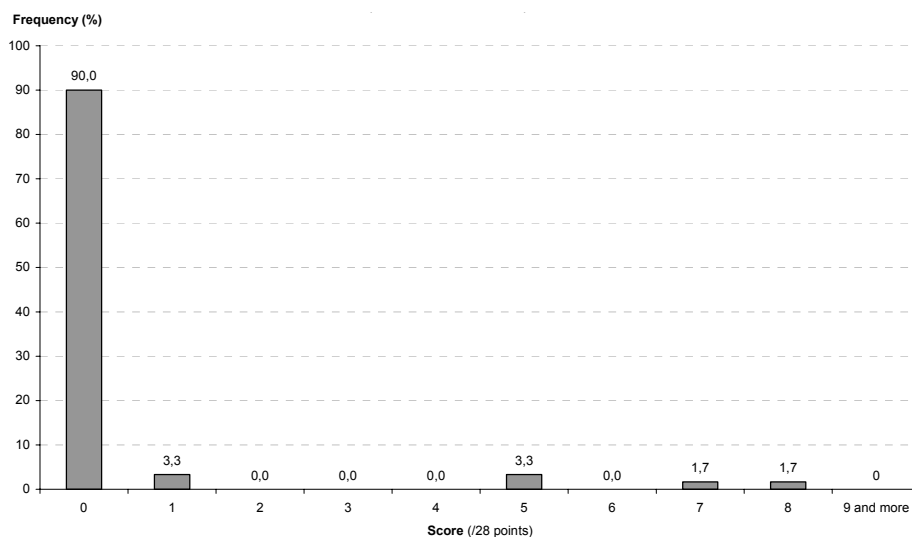
Respiratory disorders are considered as the most serious problem affecting finishing pigs worldwide. Lung lesions like pneumonia and pleuritis are observed since decades in a high proportion of fattening pigs arriving to the slaughterhouse (Cleveland-Nielsen et al., 2002; Leneveu et al., 2004). Even if the clinical signs of the disease are not evident until the mid-to-late finishing phases, it has been suggested that the disease process started very early in life (Dee, 1996). Since pathogens associated with respiratory troubles have been isolated in the upper respiratory tract of pigs as soon as weaning, contamination by the sows may be suggested (Fablet et al., 2007). Furthermore, *Mycoplasma hyopneumoniae*, a primary respiratory infectious agent was isolated from the nasal cavities of sows (Calsamiglia and Pijoan, 2000). Even if the shedding status of sows has been investigated for some respiratory pathogens, to the best of our knowledge, few data related to the health of the respiratory tract of sows are available. The purpose of the study was to assess the type and the extend of lung lesions in a small sample of culled sows in order to prepare further investigations on respiratory disorders in farrow-to-finish pig operations.

### MATERIALS AND METHODS

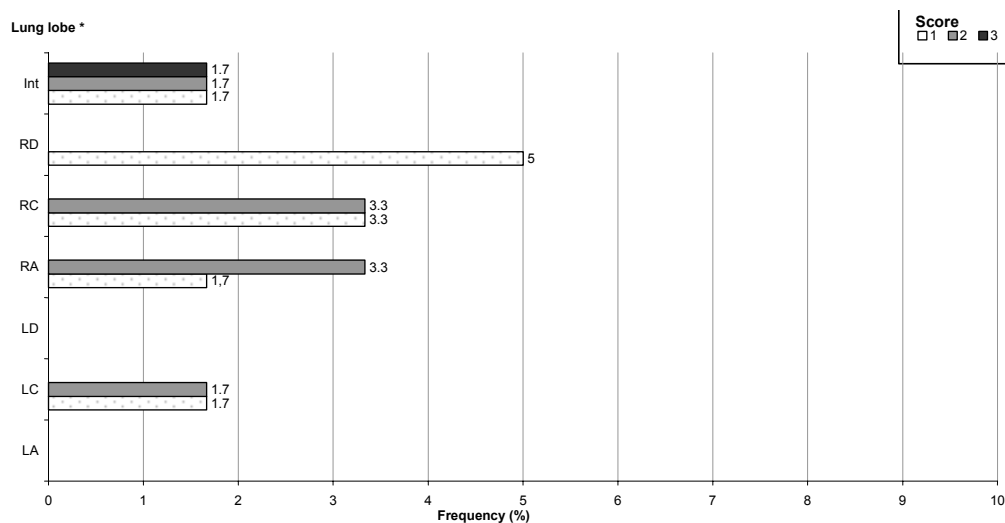
The study was carried out in a slaughterhouse in Brittany, France. The lungs of 60 sows belonging to 36 pig herds were randomly collected. Each lung was submitted to macroscopic examination for pneumonia and pleuritis. Pneumonia was scored (scale: from 0 to 28; from zero to four for each of the seven lobes according to the consolidated surface). Healing of pneumonia was also recorded. Pleuritis was evaluated (scale: from 0 to 4 according to the proportion of affected tissue). The scales used were those previously established by Madec and Derrien (1981).

## RESULTS

Distribution of pneumonia scores and affected lobes are presented Figures 1 and 2. Pneumonia was observed in 10% of the sows (6/60). The mean pneumonia score was rather low: 0.45/28 (SD=1.6) with scores ranging from 0 to 8. The right cardiac and apical lobes were the most frequently affected ones. In 11 sows, pleuritis was detected (18.3%). Healing pneumonic lesions were noticed on the lungs of 3 sows.



**Figure 1.** Distribution of pneumonia scores (60 culled sows, 2004).



\*: LA : Left Apical Lobe, LC : Left cardiac Lobe, LD : Left Diaphragmatic lobe, RA : Right Diaphragmatic Lobe, RC : Right Cardiac Lobe, RD : Right Diaphragmatic Lobe, Int : Intermediate Lobe.

**Figure 2.** Distribution of pneumonia scores according to the lung lobe (60 culled sows, 2004).

## DISCUSSION-CONCLUSION

The results of the present study indicate that lung lesions are of rather mild severity and that they are found at a low frequency in culled sows. They give a first indication for eventual further investigations of the same type carried out on a larger scale. These preliminary findings are in contrast with the high prevalence of pneumonia commonly detected at the slaughterhouse in fattening pigs. Indeed, Leneveu et al., (2004) who examined at slaughterhouse 77 087 lungs of fattening pigs belonging to 778 Brittany herds found that 74.5% of pigs were affected by pneumonia. Whether, the sows continue to shed the pneumotropic pathogens to the newborn remains to be further investigated. The finding of extended lesions in some sows does not exclude an activity of pneumotropic pathogens in the sow herd of certain farms.

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## INFECTION PATTERN IN 12 PIG FARMS DIFFERENTLY AFFECTED BY RESPIRATORY DISORDERS

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### SUMMARY

The bacteriological and serological status towards Mhp, Pm, App, Hps, Ssuis, PRRSV, PRCV and SIV was assessed in 12 pig herds differently affected by respiratory disorders. Swabs and blood samples were obtained from 30 growing pigs. Pneumonia lesions were scored at the slaughterhouse and lung tissues taken. Swabs and lung tissues were analysed by PCR to detect bacterial pathogens. Serum antibodies related to viral agents were looked for. Hps, Ssuis and Pm were commonly detected in all farms and rarely at the slaughterhouse. Mhp and App were rarely identified from live pigs. They were isolated at the slaughterhouse in every farm whatever the pneumonia score. Extended pneumonia was mainly observed in herds co-infected with PRRSV and SIV.

**Keywords:** pigs, respiratory disorders, infection pattern

### INTRODUCTION

Respiratory troubles are a worldwide issue responsible for important economic losses especially in intensive confined pig production systems. Multiple pathogens are involved in the disorders such as *Mycoplasma hyopneumoniae* (Mhp), *Pasteurella multocida* (Pm), *Actinobacillus pleuropneumoniae* (App), *Haemophilus parasuis* (Hps), *Streptococcus suis* (Ssuis), Porcine Reproductive and Respiratory Syndrome virus (PRRSV), Porcine Respiratory CoronaVirus (PRCV) and Swine Influenza Virus (SIV) (Thacker, 2001). The aim of the survey was to assess the bacteriological and serological status of 12 French herds in relation to the severity of clinical disease and lung lesions at the slaughterhouse.

### MATERIALS AND METHODS

#### Data collection

The study was carried out from May 2004 to January 2005 in 12 single-site farrow-to-finish herds affected by respiratory disorders at different levels of severity. The farms were proposed by veterinarians of the farmer organisations. In each farm, nasal (VWR International, Fontenay-Sous-Bois, France), tonsillar (VWR International, Fontenay-Sous-Bois, France), oro-pharyngeal (Orifice Medical AB, Ystad, Sweden) swabs and blood samples were obtained from 30 pigs: 10 in the post-weaning section, 10 at the beginning and 10 at the end of the finishing phase. Clinical signs of cough and sneezing were recorded in farrowing, post-weaning and finishing sections. For



the batches of the sampled finishing pigs, lungs of at least 30 pigs were collected at the slaughterhouse. Macroscopic lesions of pneumonia were scored (scale: from 0 to 28 according to the consolidated surface) (Madec and Kobisch, 1982) and samples of lung lesions were taken to the laboratory.

### Laboratory analyses

Swabs and lung tissues were examined by PCR for the detection of Mhp, App, Hps and Ssuis according to methods described elsewhere (Savoye *et al.*, 2000; Verdin *et al.*, 2000; Oliveira *et al.*, 2001; Marois *et al.*, 2004). For Pm, the PCR-test was developed in our laboratory. All sera were analysed for the detection of antibodies to PRRSV, SIV (subtypes A/sw/H<sub>1</sub>N<sub>1</sub>, H<sub>3</sub>N<sub>2</sub> and H<sub>1</sub>N<sub>2</sub>) and PRCV (LDA 22, France). A pig was considered as carrier when at least one swab tested PCR positive. A farm was declared infected by PRRSV or PRCV as soon as 1 serum sample was positive. For SIV, a farm was considered infected when antibodies were detected in at least 2 pigs.

## RESULTS

Clinical signs of respiratory troubles were recorded in all farms, sneezing being the most reported one (Table 1). In 6 farms, pigs exhibited sneezing as soon as the farrowing phase. Cough was noticed in fattening sections in 8 out of the 9 farms affected by this symptom. Piglets of 3 farms showed cough outbreaks in the farrowing phase. Hps, Ssuis and Pm were identified from live pigs in the 12 farms. All herds were seropositive to PRCV. Pigs of 3 farms were seronegative to PRRSV and SIV (farms 02, 10 and 12) and those of 4 herds were seropositive to both viruses (farms 01, 04, 07, 08) (Table 1). Mean scores of pneumonia ranged from 0.9 to 10.4. Mhp and Pm were the most frequent bacteria detected in lung tissue showing pneumonia (Table 2).

**Table 1.** Respiratory symptoms, bacteriological results of swab samples from live pigs and serological profiles (12 pig farms, 30 pigs/farm, 2004–2005)

Farm	Clinical signs <sup>1</sup>		Number of positive pigs					Serological profile <sup>2</sup>		
	Cough	Sneezing	Mhp	Pm	App	Hps	Ssuis	PRRS	SIV	PRCV
01	PW/F	Far/PW/F	0	4	2	29	13	+	+	+
02	F	PW/F	0	28	0	30	28	-	-	+
03	-	Far/PW	0	21	11	25	21	+	-	+
04	F	Far/PW/F	2	28	0	30	19	+	+	+
05	-	F	0	13	0	30	19	+	-	+
06*	F	PW	0	20	0	17	17	+	-	+
07**	Far/F	Far/PW/F	3	26	0	30	18	+	+	+
08	F	Far/PW/F	0	8	0	28	2	+	+	+
09	Far/PW/F	F	0	16	0	30	2	+	-	+
10	Far	Far/PW/F	1	19	0	30	13	-	-	+
11	-	PW	1	9	0	28	30	-	-	+
12	F	PW	0	8	2	29	0	-	+	+

\* (10 growers and 10 market-aged pigs) \*\* nasal swabs

1: Far: Farrowing section, PW: Post weaning section, F: Fattening phase

2: +: positive; -: negative

**Table 2.** Pneumonia scores and PCR results from lung tissues for 12 farms (+: positive; -: negative)

Farm	Pneumonia score (/28) ( $\sigma$ )	mean	Pathogen detection in lung lesions				
			Mhp	Pm	App	Hps	Ssuis
01	6.4 (5.1)		+	-	-	+	-
02	2 (3.0)		+	+	-	-	+
03	2.8 (3.4)		-	-	-	-	-
04	3.7 (3.9)		+	+	-	-	-
05	4.4 (5.8)		+	+	-	-	-
06	9.6 (4.2)		+	-	+	+	-
07	10.1 (6.3)		+	+	-	-	-
08	10.4 (6.7)		+	+	-	-	-
09	2.1 (3.7)		+	+	-	-	-
10	3.3 (4.0)		+	-	-	-	-
11	0.9 (2.5)		+	-	-	-	+
12	5.4 (4.0)		+	+	-	-	-

## DISCUSSION AND CONCLUSION

According to our results, respiratory symptoms are widely distributed in pig farms, sneezing being the most prevalent. Cough tended to be limited to the fattening sections. The results of the study showed that the upper respiratory tract of live pigs was commonly contaminated with Hps, Ssuis and Pm. Despite the high prevalence of Hps and Ssuis at the farm level, both pathogens were not systematically recovered from lung lesions. In contrast, although Mhp was rarely detected from live pigs at the farm stage, the pathogen was isolated at the slaughterhouse from lung lesions of pigs coming from all the farms, whatever the pneumonia scores. Although some bacterial pathogens associated with respiratory disorders seem to constitute a common flora in these farms, the viral patterns and intensity of lung lesions differed among herds. Severe lesions of pneumonia were mainly observed in herds co-infected with PRRS and SIV. Obviously, not only bacterial pathogens but also viral ones and other factors related to farming practices must be considered to properly understand the onset and severity of respiratory disorders in pig herds. In addition, the way we used to sample and to process material for pathogen detection in live pigs, especially for Mhp, needs further improvements (mainly sensitivity of detection in live pigs).

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge the farmers. The project was financially supported by Boehringer Ingelheim, Fort Dodge S.A., Intervet S.A., Pfizer, Schering-Plough Vétérinaire, the “Conseil Régional de Bretagne” and the “Comité Régional Porcin”.

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**THE DETECTION OF *M. HYOPNEUMONIAE*, *P. MULTOCIDA*,  
*S. SUIIS*, *H. PARASUIIS* AND *A. PLEUROPNEUMONIAE* IN PIGLETS  
AT WEANING IN 5 FARROW-TO-FINISH FARMS**

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**SUMMARY**

A survey was carried out to assess the contamination of piglets by *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, *Streptococcus suis* and *Haemophilus parasuis* in 5 farrow-to-finish pig farms. At weaning, in each farm, a sample of 60 piglets belonging to one batch was randomly selected and submitted to nasal, tonsillar and oropharyngeal swabs. All samples were analysed with PCR to detect the pathogens. The study indicates that Hps, Pm and Ssuis are commonly found in the upper respiratory tract of young piglets. Contamination by Mhp and App seems to be less detected at this stage of rearing according to the methods used.

**Keywords:** pneumotropic pathogens, pigs, weaning, contamination

**INTRODUCTION**

Pathogens associated with lung lesions of pigs are numerous and diverse. Among bacteria, *Mycoplasma hyopneumoniae* (Mhp), *Pasteurella multocida* (Pm), *Actinobacillus pleuropneumoniae* (App), *Streptococcus suis* (Ssuis) and *Haemophilus parasuis* (Hps) are the pathogens the most frequently involved in respiratory disorders (Thacker et al., 2001). Numerous studies were carried out to describe pig contamination during post-weaning and/or fattening periods. Nevertheless, the respiratory flora of pigs and its evolution at these growing stages depends on the flora acquired in the early phase of the piglet's life. To the best of our knowledge, few data related to the acquisition process of these pathogens during the lactation phase are published. Therefore, the aim of the present survey was to assess the contamination of piglets by Mhp, Pm, App, Ssuis and Hps at weaning in 5 farrow-to-finish French pig farms differently affected by respiratory disorders.

**MATERIALS AND METHODS**

**Study design**

The farms were ranked on a scale (from 1 to 5) according to their score on clinical criteria at the post weaning and fattening stages as well as on the lesions at slaughter. A description of the respiratory status of the farms is given Table 1. At weaning, in the five farms, a sample of 60 piglets belonging to one batch was randomly selected. Each piglet was submitted to nasal (VWR

International, Fontenay-Sous-Bois, France), tonsillar (VWR International, Fontenay-Sous-Bois, France) and oro-pharyngeal swabs (Orifice Medical AB, Ystad, Sweden). After sampling, swabs were placed in peptone water and shipped to the laboratory.

**Table 1.** Description of lung lesions observed at the slaughterhouse on a sample of pigs (5 farrow-to-finish pig farms, France, 2004–2005)

Farm	Sample size	Pneumonia	Pleuritis	Rhinitis
		Mean score (/28 points) (SD)	Percentage of lung lesions (%)	Mean score (/18 points) (SD)
1	50	0.2 (0.6)	4	2.9 (2.8)
2	36	3.5 (3.8)	11.1	4.1 (2.9)
3	55	1.8 (2.0)	1.8	6.3 (3.0)
4	40	11.8 (6.9)	27.5	6.3 (3.4)
5	47	10.7 (5.7)	42.5	5.3 (3.4)

### Laboratory analyses

All samples were analysed in our laboratory with PCR tests aiming at the detection of Mhp, App, Hps and Ssuis (Savoye *et al.*, 2000; Verdin *et al.*, 2000; Oliveira *et al.*, 2001; Marois *et al.*, 2004). For Pm, the PCR test used was specially designed and performed by our laboratory team. A pig was considered as carrier as soon as one swab tested PCR positive.

## RESULTS

Weaning age varied from 21 to 28 days (Table 2). Hps and Pm were identified in all farms with frequency rates ranging from 35 to 100% for Hps and 8.3 to 100% for Pm. Ssuis was detected in 4 farms with a frequency of 1.7%, 60% and 65% (2 farms), respectively. Mhp was detected in only one pig. App was identified in one herd at a low frequency (6.7%) (Table 2).

**Table 2.** Contamination of piglet by Hps, Pm, Ssuis, Mhp and App at weaning (5 farms, France, 2004–2005)

Farm	Mean weaning age (days)	Frequency of PCR-Positive piglets (%)				
		Hps	Pm	Ssuis	Mhp	App
1	20	95	55	1.7	0	0
2	29	81.7	8.33	65	1.7	0
3	20	85	63.3	0	0	6.7
4	27	35	41.7	60	0	0
5	28	100	100	65	0	0

## DISCUSSION AND CONCLUSION

From this small-scale survey, it appears that most of the pneumotropic pathogens, especially Hps, Pm and Ssuis can early colonise the upper respiratory tract of piglets, suggesting an early contamination and a probable seeder role of the sows. The study also indicates that whatever the severity of expression of respiratory disorders in pigs at the later stages in the herds, Hps, Pm and Ssuis were commonly found in the young piglets. Hps is described as one of the earliest agent colonising the respiratory mucosa of piglets after birth (Rapp-Gabrielson, 1999). Even if Mhp was detected at 4 weeks of age, detection rate of this pathogen was rather low according to the sampling procedure and the laboratory methods used. App was rarely identified at this stage of life. Dealing with these results, the respiratory flora of pigs of 3 to 4 weeks of age seems to be diverse, Hps, Pm and Ssuis belonging to the common bacterial flora of the upper respiratory tract. The suckling period appears to be a decisive step in the acquisition of respiratory pathogens. Beside a qualitative detection of the infectious agents, a quantitative approach should be relevant in order to better describe the contamination load to which the piglets are exposed. Furthermore, the results of the present study suggest considering both the dynamics of the major specific-pathogens involved as well as the management and husbandry conditions prevailing on the farms along the rearing period, when looking for the risk factors of respiratory disease complex in pigs.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge the farmers. The project was financially supported by Boehringer Ingelheim, Fort Dodge S.A., Intervet S.A., Pfizer, Schering-Plough Vétérinaire, the “Conseil Régional de Bretagne” and the “Comité Régional Porcin”.

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## **EFFECT OF BROVAGLUKIN ON VALUES OF BLOOD AND PRODUCTIVE QUALITIES IN PREGNANT GILTS**

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### **SUMMARY**

The effect of the complex preparation brovagulkin on some biochemical indices of blood, humoral and cellular factor of the body protection has been studied. It has been stated that brovagulkin, 4 ml per 10 kg live weight, stimulates the productive qualities of piglets produced by the gilts of the experimental group, improves the durability of the litter.

**Key words:** brovagulkin, blood, pregnant, gilts, suckling, piglets, resistance, biochemical values

### **INTRODUCTION**

The most important environmental factors that result in significant interior changes in the body and effect the intensity of animal growth and development are different feeding stuffs and biologically active substances [2,4]. The problem of the balanced rations for animal feeding is topical, especially from the point of view of mineral nutrition [1]. The deficiency of macro and microelements and BAS in the gilt ration leads to the lowering of resistance and productivity [5]. So, the use of complex preparations is the necessary condition to prevent animal diseases, to increase immunological status and productive potential of animals.

### **OBJECTIVE**

The aim of the experiment was to study and to develop new ways to improve animal keeping hygiene, to promote the increase in animal resistance and productivity and to keep the piglets healthy in their critical periods of growth.

### **MATERIALS AND METHODS**

To achieve the above objective 37 pregnant gilts of Large White breed were divided into two groups. The animals were of the same age, live weight and of the third pregnancy.

The gilts of the experimental group were kept in the conditions of microclimate and sanitary regime according to the technological design standards for pig-breeding farms. 18 gilts of the control group were fed by the ration that provided them with all essential elements of animal nutrition. 19 gilts of the experimental group received the same ration but they were administrated IM brovagulkin, 4ml per 10kg live weight, twice, on the 35 and 15 days before farrowing.

Brovaglugin is powder of light yellow colour, packed in flasks.

Before the administration of the preparation the content of the flask was diluted by isotonic solution. 1 ml of the solution for injection contains 100 mg Na sulphadimetotoxin, 100 mg Na sulphadiazin, 25 mg Ca ions, phosphorus ions – 12,5 mg, Mg ions – 5 mg and 4 mg cholinchloride.

The estimation of ammonia, carbon dioxide and hydrogen sulphide levels in the pigsty was carried out by the express-analyzer “Gas tester, type KI-28066, light intensity was measured by luxometer, type U-117, temperature –by the special device II-611.

The health status of the gilts and their litter was determined by the blood and blood plasma indices. For that standard reagents produced by “Lachema” (Chechia) were used. The state of immune organs in the piglets was evaluated by the common methods.

The effect of brovaglugin on haematological values of blood in the pregnant gilts was studied by morphological, biochemical and immunological methods: the level of haemoglobin was determined by FEC-56 M, leukocyte content was determined in Boryaev's chamber, the concentration of circulating immune complexes (CIC) was estimated by Grinevich's method, etc [3, 6, 8], lyzocymic activity-by Perri [8], the amount of the G and M immune classes – by Manchini [9].

## RESULTS

During the experiment some changes in the values of natural resistance in the pregnant gilts have been revealed (Table 1).

**Table 1.** Indices of humoral and cellular protection in pregnant gilts

Index	Groups		Referent Level
	Control	Experimental	
	35 <sup>th</sup> 15 <sup>th</sup> day before farrowing		
BABS, %	<u>50,1±3,8</u>	<u>49,1±2,27</u>	40–60
	45,1±2,4	48,4±1,14*	
LABS, %	<u>39,6±2,14</u>	<u>40,7±1,31</u>	30–50
	35,1±1,17	38,4±1,36*	
PAN,%	<u>27,3±0,72</u>	<u>26,8±1,18</u>	30–40
	22,6±0,80	24,3±1,12	
<b>T-lymphocytes</b>	<u>4,61±0,03</u>	<u>4,55±0,04</u>	3,1–4,5
	3,81±0,02	4,27±0,02*	
B- lymphocytes	<u>1,78±0,04</u>	<u>1,74±0,03</u>	1,0–3–5
	1,62±0,03	2,01±0,04*	



10<sup>th</sup> day 5<sup>th</sup> day before farrowing

BABS, %	$\frac{30,4 \pm 0,62}{41,2 \pm 1,10}$	$\frac{46,7 \pm 1,04^*}{36,2 \pm 0,85}$	40–50
LABS, %	$\frac{32,5 \pm 1,12}{36,4 \pm 1,08}$	$\frac{37,1 \pm 1,15^*}{39,1 \pm 0,10^*}$	30–50
PAN %	$\frac{20,1 \pm 0,21}{27,4 \pm 0,20}$	$\frac{24,8 \pm 0,31^*}{32,1 \pm 0,34^*}$	30–40
T-lymphocytes g/l	$\frac{3,48 \pm 0,03}{4,43 \pm 0,04}$	$\frac{5,04 \pm 0,04^*}{5,27 \pm 0,05^*}$	3,1–4,5
B-lymphocytes g/l	$\frac{1,74 \pm 0,03}{2,08 \pm 0,02}$	$\frac{2,10 \pm 0,04^*}{2,38 \pm 0,05^*}$	1,0–3,5

Note: the numerator indicates the values on the 35<sup>th</sup> and 10<sup>th</sup> days; the denominator indicates the values on the 15<sup>th</sup> and 5<sup>th</sup> day, respectively.

\*P < 0, 05

\*\*P < 0, 01

The analysis of the natural resistance levels (Table 1) showed the reduction of BABS level in the gilts of the experimental group. The level of BABS on the 35<sup>th</sup> day before the farrowing was not lower than 48,4±1,14%, 15<sup>th</sup> day before farrowing the above level was 46,7±1,04%, on the 5<sup>th</sup> day before farrowing it was 36,2±0,85%.

The level of LABS was trustworthy higher [P < 0, 05] as compared with that in the gilts of the control group before farrowing on the days mentioned above. The amount of lymphocytes in the blood of the gilts in the experimental group on the 35<sup>th</sup> day before farrowing was 11,2% higher than that in the control group and on the 10<sup>th</sup> day it was higher by 10,7%, the level of B-lymphocytes in the above periods of the investigation was in the range of 2,01±0,04 g/l, on the 35<sup>th</sup> day before farrowing –2,10±0,04 g/l, on the 5<sup>th</sup> day before farrowing –2,38±0,05 g/l.

It means that the shorter the period before farrowing the higher the levels under investigation. The piglets produced by the gilts of the experimental group had better indices of natural resistance as compared to the piglets born by the gilts of the control group (Table 2).

**Table 2.** Humoral and cellular levels of natural resistance in suckling piglets

Index	Investigation after birth, days		
	10	21	60
BASS %	$\frac{34,1 \pm 0,24^*}{26,4 \pm 0,31}$	$\frac{38,04 \pm 0,38^*}{28,7 \pm 1,01}$	$\frac{48,6 \pm 1,21^*}{40,1 \pm 1,12}$
LABS %	$\frac{27,8 \pm 0,09^*}{24,1 \pm 0,08}$	$\frac{36,1 \pm 1,1^*}{25,8 \pm 0,09}$	$\frac{48,7 \pm 1,2^*}{40,1 \pm 1,08}$
PAN, %	$\frac{64,5 \pm 0,41^*}{58,6 \pm 0,56}$	$\frac{70,2 \pm 0,9}{68,5 \pm 1,12}$	$\frac{74,5 \pm 0,7^{**}}{6,57 \pm 1,10}$

Note: the nominator indicates the values received in the experimental group of animals; denominator indicates the results of the control group.

\*P < 0, 05

\*\*P < 0, 01

The analysis of the data given in Table 2 shows that BABS level in the 10<sup>th</sup> day-old piglets was higher by 7, 7% as compared to the piglets of the same age in the control group, the above level in the 21-day- old piglets was higher by 9, 34% and in the 60 day-old- piglets – by 8, 5% as

compared to the control ones ( $P < 0,05$ ). The level of LABS in the piglets of the experimental group in the above periods ranged from  $27,8 \pm 0,09$  to  $48,7 \pm 1,2\%$ , PAN level was  $5,9$ – $8,8\%$  higher in 10 and 60 days old piglets than in the control group ( $P < 0,05$ – $0,001$ )

Positive changes in the immune defence have been revealed in the piglets produced by the gilts of the experimental group.

It can be proved by the lower number of piglets having symptoms of gastric disturbances (lower by 1, 5–1, 9 times) and the better durability of the piglets born.

Brovagulkin had an influence on the morphological state of the immunocompetent organs (Table 3).

**Table 3.** Live weight of immunocompetent organs in piglets

Group	Thymus weight, g	Thymus index, %	Spleen weight g	Spleen index, %
Control	$1,09 \pm 0,01$	0,172	$0,61 \pm 0,51$	0,081
Experimental	$1,15 \pm 0,01^*$	0,212**	$0,75 \pm 0,20$	0,096

Note: \* $P < 0,05$  and \*\*  $P < 0,01$

The data in Table 3 show that the use of brovagulkin stimulated the increase in the protective functions of the piglets. It can be proved by the increase in the weights of thymus and spleen and by the increase in the indices of the above organs.

Blood is one the mobile systems in which the state and the processes of metabolism in the body are reflected (Table 4).

**Table 4.** Biochemical values of blood in piglets

Group	Protein %	Calcium mg %	Phosphorus mcg %	Vitamin A, mcg %	Glucose mmol/l
Control	$5,48 \pm 0,20$	$8,91 \pm 0,22$	$4,27 \pm 0,20$	$46,8 \pm 2,01$	$3,42 \pm 0,2$
Experimental	$6,75 \pm 0,21^*$	$10,65 \pm 0,31$	$5,58 \pm 0,22^*$	$53,2 \pm 1,96^{**}$	$4,11 \pm 0,2^*$
Referent index	7–8,4	10–14	40–60	30,0–70,0	4,5–10,0

Note \* $P < 0,05$ ; \*\* $P < 0,01$  in ratio to the control.

It can be seen from Table 4 that biochemical indices of the gilts from the control group are significantly different from the ones of the experimental animals. The level of protein in the animals of the control group was lower by 8,9%, calcium – by 15,9%, phosphorus – by 23,5%, vitamin A – by 12,1%, glucose – by 16,8% than in the experimental group.

The analysis of the data received shows that the use of brovagulkin affected the productive properties of the gilts under investigation and the litter produced by the above gilts (Table 5).

**Table 5.** Reproductive qualities of gilts

Group	Multi foetus, number of piglets	Large size of foetus	Number of piglets born	Milking quality, kg
Control (n=18)	10,6±0,21	1,21±0,08	173	58,1±1,40
Experimental (n=19)	11,7±0,15*	1,32±0,05	220	62,8±1,34**

Note: \*P<0, 05; \*\* P<0, 01

It can be seen from Table 5 that the gilts of the experimental group had better indices of reproductivity than the gilts of the control group.

The experimental gilts exceeded the control ones on the number of piglets born by 27, 1%, the largeness of foetus by 9, 0% and in multifoetus –by 8, 1%.

During the experiment the positive effect of brovaglukin on the growth of piglets was revealed (Table 6).

**Table 6.** Growth intensity, durability, and morbidity of piglets

Group	Age of piglets, days			Morbidity, %	Durability, %
	10	21	60		
Control	2,63±0,20	4,90±0,02	15,7±0,2	21	90,4±0,30
Experimental	2,95±0,18	5,11±0,03*	16,8±0,3*	12	94,8±0,34**

Note: \*p<0, 05; \*\*p<0, 01

During the suckling period the piglets produced by the gilts of the experimental group grew more intensively: on the 10<sup>th</sup> day the growth intensity index was higher by 12,1%, by the 21<sup>st</sup> day – by 4,2% and on the 60<sup>th</sup> day of their life – by 7% as compared to the piglets born by the gilts from the control group. The average daily weight gains were 279g, it is by 6, 1% higher than the values in the control group

## CONCLUSION

On the basis of the data received during the experiment it has been stated that brovaglukin provides the organs of the gilts with the essential mineral substances that intensify metabolic processes, stimulate body natural resistance and productive potential of the gilts. The combination of sulphanilamides inhibits the growth and reproduction of microorganisms. Phosphorus has general stimulating action and activates the fermentation processes, Mg promotes protein and carbohydrate metabolism, cholinchloride regulates phosphorous and lipid metabolism. The use of the preparation in the dose 4mg per 10kg of live weight has positive influence on the reproductive qualities of pregnant gilts, on the productivity and durability of the piglets produced by the gilts.

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## THE INFLUENCE OF PREGNANT SOWS' MOVEMENT RESTRAINT ON LYMPHOCYTE PROLIFERATION IN SOWS AND PIGLETS

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### OBJECTIVE

The movement restraint is a strong stress incentive among the animals, which may influence the immunologic reactions. Currently valid standards concerning husbandry of pregnant sows allow movement restraint that delimits basic behavioural reactions and also may influence the animals' health.

### METHODS

The aim of the study was to define the influence of movement restraint usually used in pig husbandry on lymphocyte proliferation. In the experiment we used pregnant pigs:

- I – no movement restraint (1–100 day from insemination pigs were housed in group crates, 100–135 day in stroll parturition crates)
- II – movement restraint (1–28 day from insemination pigs were housed in individual crates, 30–100 day group crates, 100–135 day in individual parturition crates)
- III – movement restraint through the whole parturition period (1–100 day from insemination pigs were housed in individual crates, 100–135 day in individual parturition crates).
- Blood were taken from *vena cava*:
- sows – 30, 98, 100, 135 day from insemination
- piglets – 3, 7, 21 day of live

Mitogen-induced lymphocyte proliferation was used as an *in vitro* index of cellular immune function. Concanavalin A, pokeweed mitogen and phytohemagglutinin were used in the lymphocyte proliferation assay.

### RESULTS

On the 99th day the proliferation results were comparable to all groups and on the 101st (after inserting into parturition crate) showed the highest immunity suppression in the 2nd group. A similar result could be observed on the 30th day of the experiment. The prenatal stress resulted in deterioration of lymphocytes' proliferation of piglets from the 3rd group at the age of 3, 7 and 21 days.

### **CONCLUSIONS**

The results unequivocally indicate the possibility of influence of prenatal stress on the immunological system's ontogenesis, which may cause higher susceptibility on diseases during the fattening period.

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## EFFECT OF ORGANIC REARING SYSTEM ON PORK QUALITY AND PREFERENCE

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### SUMMARY

Although organic meat production is only a small proportion of the total meat production in EU, it is increasing in size to take demands of certain market segments into account. The current Lithuanian production systems for pig meat are optimized for high production and uniform product quality. However, to adapt to sustainability goals concerning animal welfare and environment impact and to improve the competitiveness of Lithuanian meat, the conventional systems must be continuously evaluated.

This study concerns scientific studies results comparing indoor and outdoor pig finishing systems. There are some real differences in pork quality noted in the literature. However, differences in pork quality vary among the different environments that were investigated. Consumer perception is such that when they enter a retail environment they are willing to buy pork products with social assurances.

**Keywords:** pig finishing systems, pork quality; pork quality measures

### INTRODUCTION

The consumers of organic pork associate organic pig production with a high standard of animal health and welfare and with a high degree of food safety and quality. The development of organic pig production in the European Union is affected by EU-regulation 1804/1999 (Anon., 1999). This EU-regulation, which was implemented in 2000, provides a framework for animal health and welfare management in organic pig production.

At present, only a few organic animal husbandry farms exist within the Lithuania, but the market is developing and a major expansion in organic production is anticipated. Although, organic pig production is a small-scale system compared with organic milk and beef production. This weaker development seems most likely to be due to difficulties for pig producers to comply with the organic standards, which impose comparatively more pronounced changes in the way of production than e.g. in ruminant production systems. Pigs should have access to roughage and to grazing in the summer period although finishers can be kept in barns if access to an outdoor rum. Information on the optimal methods to achieve biologically and economically efficient production under organic standards is urgently needed. In this study overview of scientific studies focusing on rearing system influence on pork quality and preference has been done.

## PORK QUALITY IN AN INDOOR AND OUTDOOR FINISHED PIGS

Although benefits and options of keeping pigs in free range systems have been described in different European countries (Van der Wal, 1993; Watson & Edwards, 1997), this production method is not very common in some EU countries. There are some advantages and disadvantages shown in Table 1. Post-mortem pH and water holding capacity may be reduced in outdoor pigs (Warriss et al., Enfält et al., 1997). A comparison of the effects of environmental housing systems on pork colour and sensory characteristics are included in Table 1. Loins from outdoor reared pigs had a lower ultimate pH, higher drip loss, and higher Warner-Bratzler shear force values (Enfält et al., 1997) than loins from indoor finished pigs during the winter months in Sweden. Meat from outdoor finished pigs also had more lactate and crude protein, higher glycolytic potential, less intramuscular fat, and less water (Enfält et al., 1997).

Several researchers have found no differences in pork eating quality measurements comparing pork from indoor and outdoor reared pigs (van der Wal, 1991; Barton-Gade and Blaabjerg, 1989). Beattie et al. (2000) reported that pigs from enriched environments produced pork with greater tenderness than pigs rose in barren environments. Jonsäll et al. (2001) reported that ham from outdoor reared pigs was less juicy and acidulous than ham from indoor reared pigs ( $P < 0.05$ ) but no differences were found in tenderness, odour intensity, or meat taste between the indoor and outdoor reared groups. Maw et al. (2001) reported that pigs housed on straw bedding produced bacon with a stronger fried meat flavour than bacon from pigs housed on concrete or slats ( $P < 0.05$ ). Bacon from straw-bedded pigs was darker in colour than bacon from pigs raised either on concrete or slatted flooring (Maw et al., 2001). Other researchers have found no effect of physical activity on sensory qualities of cuts from the ham and loin (Petersen et al., 1997; van der Wal et al., 1993; Essén-Gustavsson et al., 1988), but the degrees of exercise and enrichment of the environments varied.

A summary of carcass measurements, colour scores, and sensory characteristics comparing loins of pigs raised in indoor and outdoor finishing systems is included in Table 2. Pigs finished outdoors during the warm months had more back fat at the last rib than pigs finished indoors. For the group processed in March, the outdoor-born pigs had more back fat at the 1st and last rib than the indoor-born group. Also, outdoor-reared pigs had more back fat at the last rib but less marbling on the loin eye.

Outdoor born and finished pigs had lower  $L^*$  and higher  $a^*$  values than indoor born and finished pigs ( $P < 0.05$ ). Minolta  $a^*$  values were highest for the pigs born and finished outdoors, indicating a redder colour of the loin muscle.

Chops from the outdoor-born pigs (processed in July) had more desirable sensory panel scores for flavour intensity (Table 2) and lower shear force values, indicating more tender meat. However, no differences were detected in sensory panel scores or shear force values of loins from pigs processed in March. Loins from both groups had acceptable shear force values that would be considered very tender by most consumers (Miller et al., 2001).

Other studies compared each of the following finishing systems: indoors on concrete slats, indoors in converted poultry buildings on deep bedding with curtain sides, outdoors on a dirt lot, and outdoors on alfalfa pasture. Results from these experiments showed that pigs finished in alternative systems have similar carcass and pork quality characteristics compared to pigs finished in a conventional indoor system (Jessica G. et al., 2003). Outdoor-housed pigs grew faster than indoor-housed pigs during the warm months (Gentry et al., 2002a). Seasonal differences in growth patterns may exist with outdoor finished pigs. Outdoor-reared pigs had heavier carcass weights, less back fat at the last rib, larger loin eye area, and higher loin marbling scores ( $P <$



0.05, Gentry et al., 2002a). In addition to growth and pork quality advantages, loins from the outdoor-finished pigs had higher scores for initial juiciness (more desirable) and less off-flavour ( $P < 0.05$ ) as evaluated by a trained sensory panel. Overall, outdoor or deep-bedded systems may increase growth rates of pigs if suitable land area and resources are available, but pork quality of loins will be similar for pigs finished in either conventional or alternative systems.

Few differences were detected in loin muscle quality (colour or pH) among the experiments. Shear force values were higher (tougher) for outdoor finished pigs in three experiments but lower (more tender) in two experiments. Again, results in loin muscle quality between indoor and outdoor (or alternative) housing systems are variable (Jessica G. et al., 2003). Many other factors could be confounding results such as environmental conditions, management, diet, genetics, or others. Swine producers should consider all of these factors when choosing a production system to best suit their environment.

**Table 1.** Pork loin measurements of alternative systems for finisher pigs.

Authors	Year	Systems	Alternative vs. Conventional <sup>a</sup>		
			L* <sup>b</sup>	pH	Shear force <sup>c</sup>
Warriss et al. (UK)	1983	Non-intensive (outdoor) vs intensive	-10%	NS	-
van der Wal (Netherlands)	1991	Free range vs indoor	NS	NS	NS
van der Wal et al. (Netherlands)	1993	Straw vs. concrete	NS	NS	NS
Sather et al. (Canada)	1997	Outdoor vs. indoor-winter	NS	NS	NS
		Outdoor vs indoor-summer	-3.0%	NS	+18%
Enfalt et al. (Sweden)	1997	Outdoor vs indoor	+5.8%	-1%	+12%
Beattie et al. (UK)	2000	Enriched vs barren	-	-	-9%
Gentry et al. (Texas, USA)	2002	Outdoor pasture vs. slats, birth environment	NS	NS	NS
		Outdoor pasture vs. slats, rearing environment	-4.7%	NS	-9.1%
Olsson et al. (Sweden)	2003	Organic vs conventional	NS	-0.9%	+12.1%

<sup>a</sup>A positive value indicates an increase for the alternative production system and a negative value indicates a decrease for the alternative production system compared to the indoor system.

<sup>b</sup>A decrease in L\* indicates a darker coloured loin. L\* values range from 1 to 100 with 1 = pure black and 100 = pure white.

<sup>c</sup>A higher shear force value indicates tougher meat.

**Table 2.** Environmental effects on pork carcass measurements, loin shear force and sensory characteristics over seasons (Gentry et al., 2002b; Gentry et al., 2003).

Processing date	July				March			
	Birth		Rearing		Birth		Rearing	
Measure <sup>c</sup>	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
No. of pigs	12	12	12	12	12	12	12	12
1st rib BF, cm	3.8	3.7	3.8	3.7	3.9 <sup>b</sup>	4.3 <sup>a</sup>	4.1	4.2
Last rib BF, cm	2.5	3.1	2.8 <sup>b</sup>	3.6 <sup>a</sup>	2.1 <sup>b</sup>	2.5 <sup>a</sup>	2.2	2.4
LEA, cm <sup>2</sup>	49.7 <sup>b</sup>	54.6 <sup>a</sup>	51.3	52.9	43.5	40.2	42.7	40.9
Marbling score <sup>d</sup>	2.3	2.7	2.5	2.5	1.3	1.3	1.5 <sup>a</sup>	1.1 <sup>b</sup>
Shear force, kg	2.1	2.1	2.2 <sup>a</sup>	2.0 <sup>b</sup>	2.2	2.1	2.1	2.1
L* <sup>e</sup>	49.5	49.2	50.5 <sup>a</sup>	48.1 <sup>b</sup>	53.6	55.0	54.9	53.8
a* <sup>f</sup>	1.4 <sup>b</sup>	2.4 <sup>a</sup>	1.6	2.2	4.2 <sup>b</sup>	5.0 <sup>a</sup>	4.2 <sup>b</sup>	5.0 <sup>a</sup>
b* <sup>g</sup>	10.2 <sup>b</sup>	10.9 <sup>a</sup>	10.5	10.7	8.9 <sup>b</sup>	9.8 <sup>a</sup>	9.2	9.6
Flavour intensity <sup>h</sup>	6.1 <sup>b</sup>	6.5 <sup>a</sup>	6.2	6.3	5.9	5.9	6.0	5.9

<sup>a,b</sup>Means in the same row within a main effect (indoor vs outdoor) with different superscripts differ ( $P < 0.05$ ).

<sup>c</sup>No treatment effects were observed for firmness, sensory panel juiciness, or sensory panel tenderness scores.

<sup>d</sup>Marbling scores were measured on the *Longissimus* muscle at the 10th rib interface on a scale of 1 to 10, 1 = devoid and 10 = moderately abundant or greater.

<sup>e</sup>Minolta L\* values range from 1 to 100 with 1 = pure black and 100 = pure white, a lower L\* value indicates a darker colored pork chop.

<sup>f</sup>Minolta a\* values represent red to green colours with a higher value indicating more red colours

<sup>g</sup>Minolta b\* values represent yellow to blue colour with a higher value indicating more yellow.

<sup>h</sup>Scores for pork flavour intensity range from 1 to 8 with 1 = extremely bland and 8 = extremely intense pork flavor.

## CONCLUSIONS

It can be concluded, that results comparing indoor and outdoor pig finishing systems have been variable. Some reasons for this variation include differences in pig birth environment, seasonal effects, and quality of ground or bedding surfaces. Improvement in pig's behaviour, performance and meat quality characteristics may be substantial when the difference in housing conditions is substantial. A possible advantage for outdoor rearing may be linked to increased a\* values and decreased L\* values of the loin muscle. Darker coloured pork is more desirable for export markets because of the increase in water holding capacity. A careful economic analysis should be conducted to determine if increased back fat and feed: gain that is associated with outdoor finished pigs could be offset by higher market prices for meat products from pigs finished in an outdoor environment. Alternative nutrition research could lead to decreased back fat levels of outdoor finished pigs. If consumers are willing to pay more for products that are produced as "sustainable", "natural", or others, then these production systems could be very successful in the future.

There are some real differences in pork quality noted in the literature. However, differences in pork quality vary among the different environments that were investigated. Consumer perception

is such that when they enter a retail environment they are willing to buy pork products with social assurances. In some cases, consumers may believe the alternative pork products will taste better. It can be concluded clearly that alternative products do not taste worse than conventional products. Pork produced from pigs born and reared outdoors was equal to or better than pork from conventional systems under some circumstances in our experiences.

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## AIR-BORNE MICROBE NUMBERS IN DIFFERENT HOUSING CONDITIONS OF REARING PIGS

**Pavičić, Ž., Balenović, T., Popović, M., Valpotić, I., Biuk-Rudan, N., Valpotić, H. and Ekert Kabalin, A.**

### SUMMARY

The aim of this investigation was to determine the degree in which different housing systems and population size, with monitoring of primary microclimatic conditions, influence the number of airborne microorganisms in piglet nurseries. The investigation extended through three production cycles totaling 150 days in two different nurseries with full capacity of piglets per turn. Nursery A was 180 m<sup>2</sup> in size equipped with 16 pens with full floors and bedding area of 8 m<sup>2</sup> estimated for 20 piglets. Nursery B was 280 m<sup>2</sup> in size equipped with 60 one story cages with 3 m<sup>2</sup> of slated floors estimated for 10 piglets. Fifteen air samplings were performed in equal weekly intervals in every 45-day turn with the use of SAS 100<sup>TM</sup> (PBI International, Italy) device and Testo and Dräger (Germany) device for the control of microclimatic conditions. Air was sampled onto growth medium for the isolation of mezophylic, hemolytic, coliform bacteria and fungi. The numbers of bacteria and fungi were determined in the laboratory with standard analytical methods. According to acquired results significantly higher microbe numbers were recorded in nursery A in relation to nursery B. However, total microbe numbers in both nurseries did not exceed 10<sup>4</sup> CFU/m<sup>3</sup> ensuring beneficial conditions to overall piglet health status. The use of bedding can cause an increase of airborne microbes in the nursery but by achieving optimal microclimatic conditions and acceptable number of animals per floor space it does not negatively affect production results and welfare of weaned piglets.

**Key words:** piglets, nursery, floor and cage housing system, airborne microbes

### INTRODUCTION

Pig welfare in intensive production is significantly dependant on technological housing solutions, which along with animal numbers influence hygienic air quality. Therefore, beside control of primary microclimatic conditions (temperature, humidity, air flow speed and harmful gases) the investigation of total number and species of microbes in pig facilities is of utmost importance hence the air can serve as a reservoir of primary and potentially pathogenic microbes significant in the etiology of infectious and allergic diseases (Wathes, 1994). Many studies have been published recently regarding air hygiene in pig facilities with the use of current devices like SAS 100<sup>TM</sup> (Pavičić et al., 2006). Nonetheless, maximum concentrations of microbe numbers allowed have not been standardized to this day, mostly because of different housing systems and population density (Baekbo, 1998; Pavičić et al., 2006). Therefore, every study from this field represents a reliable contribution for establishing reference values in individual technologic housing systems, most notably with current measuring methods and prescribed animal numbers per unit of housing space.

## MATERIALS AND METHODS

The investigation was conducted through three production cycles totaling 150 days in two different nurseries with full piglet capacities per turn. Nursery A was 180 m<sup>2</sup> in size equipped with 16 pens with full floors and bedding area of 8 m<sup>2</sup> estimated for 20 piglets. Nursery B was 280 m<sup>2</sup> in size equipped with 60 one story cages with 3 m<sup>2</sup> of slated floors estimated for 10 piglets. Air exchange in both facilities was based on negative pressure ventilation. Fifteen air samplings were performed with SAS 100<sup>TM</sup> (PBI International, Italy) device in each 45 day turn in identical weekly intervals. Samples were taken from 9 diametrically different locations in the level of animal biozone. Nutrient agar, blood agar and Sabourdaud maltose agar was used for the isolation of mezophylic, hemolytic and coliform bacteria, respectively. After the incubation period the colonies were counted with an electric counter and the number obtained was expressed in m<sup>3</sup> (CFU/m<sup>3</sup>) of air. Isolated bacteria and fungi were identified by microscope and API tests (Bio Mérieux, France). Control of primary microclimatic conditions: temperature, relative humidity, airflow speed and ammonia concentration was performed after every air sampling with portable digital devices (Testo and Dräger, Germany). The acquired data was subjected to basic statistical analysis with Statistica 7.1 (StatSoft Inc., 2005) software, and eventual significant differences in average bacteria and fungi numbers between two nurseries were recorded. Microclimatic parameters were calculated as mean values.

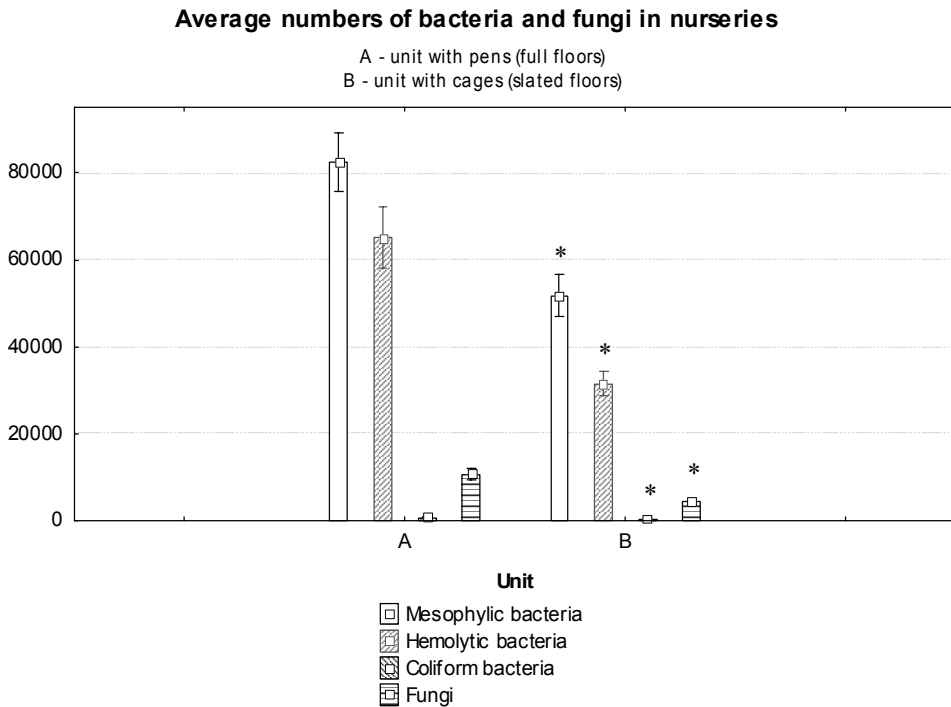
## RESULTS

Average numbers of bacteria and fungi in two different nurseries during the entire trial period is presented in Table 1 and Graph 1.

**Table 1.** Average numbers of bacteria and fungi in nurseries during the trial

Microbes	Trial period					
	Nursery A			Nursery B		
	Mean	±	SD	Mean	±	SD
Mesophylic bacteria	82360,00	±	6742,315	51742,22*	±	4807,924
Hemolytic bacteria	65104,44	±	7050,595	31442,22*	±	2777,711
Coliform bacteria	746,00	±	134,171	422,22*	±	67,044
Fungi	10678,22	±	1336,499	4517,78*	±	630,760

\* Statistically significant lower value (significance level  $p < 0,01$ ) relative to value recorded in the other facility



**Graph 1.** Average numbers of bacteria and fungi in nurseries during the trial.

\* Statistically significant lower value (significance level  $p < 0,01$ ) relative to value recorded in the other facility

Average temperature in both facilities ranged from  $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$  in the first 14 days to  $21^{\circ}\text{C} \pm 1^{\circ}\text{C}$  in the rest of the piglet production cycle. Average relative humidity in both facilities was within optimal values from 65–70%, airflow speed was 0,2 m/s, and ammonia concentration was 10 ppm.

## DISCUSSION

Breeding of weaned piglets can be conducted the classic way on full floors with bedding or in cages, during which both housing types can have a different effect on hygienic air quality in the nursery. Well-known stable microorganisms such as gram-positive and negative bacteria along with environmental saprophytes like fungi define hygienic air quality (Kiekhaefer et al., 1995). Although overall numbers of mesophylic bacteria represent the basis of hygienic air quality, we selected a greater range of selective growth medium, as to acquire a more detailed insight into microbiologic air composition in both types of nurseries. According to the obtained results a significantly higher number of individual microbes are observed in nursery A in relation to nursery B. In so doing, the number of hemolytic bacteria during the production cycle varied

proportionally with the number of mesophilic bacteria, and the number of coliform bacteria was substantially lower than other microbes, which could be attributed to their weak survival capability in the air (Zucker and Müller, 2002). As potential endotoxin carriers, the gram negative bacteria represent a very important group of microbes that can negatively affect animal health so it is recommended that their number does not exceed  $10^3$  CFU/m<sup>3</sup> air (Clark et al., 1983), which is in agreement with our investigation. The greatest difference in results between the two facilities was in total number of fungi, which was 57, 70% higher in nursery A. The increase in fungi and other microbes in nursery A needs to be addressed through the use of bedding, which was identified as a cause in similar investigations in the fattening unit (Bækbo, 1998). However, total microbe numbers in both units did not exceed  $10^4$  CFU/m<sup>3</sup>, which certainly represent optimal values in pig production (Donham, 1989). Having in mind other microclimatic parameters that were within reference values in both housing systems and the losses that were within acceptable 4% it can be concluded that the environment was beneficial to animal health (Fišer, 1970).

### CONCLUSION

Total number of microbes in the nursery air is closely related to the housing system. The use of bedding in nurseries can be the cause of an increase in microbe numbers in relation to housing systems without bedding. However, this number does not have to exceed values that may influence an increase in piglet losses, considering that primary microclimatic parameters and floor space per piglet are within optimal limits.

### ACKNOWLEDGMENTS

Results of the present study are a part of scientific project "Influence of biotechnological measures on pig health, reproduction and welfare" supported by the Ministry of Science, Education and Sports of the Republic of Croatia.

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## PRELIMINARY INVESTIGATION ON THE INFLUENCE OF AIR QUALITY ON THE INCREASE OF PIGLET LOSSES IN THE FARROWING UNIT

**Pavičić, Ž., Balenović, T., Popović, M., Valpotić, I., Tofant, A., Valpotić, H. and Ekert Kabalin, A.**

### SUMMARY

The study investigated air quality in the farrowing unit with increased piglet losses (farrowing unit A) by comparison of the obtained results of primary microclimatic values and total airborne microbe numbers to the results acquired from another farrowing unit (farrowing unit B) that operated in strictly controlled conditions with current technology and had optimal air quality. In each facility the investigation extended through two production cycles with full capacity of sows per turn. Farrowing unit A used outdated technology and was 270 m<sup>2</sup> in size equipped with 40 pens, while farrowing unit B used current technology and was 370 m<sup>2</sup> in size equipped with 60 farrowing pens. Seven air samplings were performed in every 28-day turn with the SAS 100<sup>TM</sup> (PBI International, Italy) air collector, and primary microclimatic conditions were monitored with Testo and Dräger (Germany) portable digital instruments. Air was sampled onto corresponding growth medium and the obtained results of microbe numbers were subject to statistical analysis. According to the acquired results a significantly larger number of individual microbe species was recorded in farrowing unit A in both production cycles in relation to farrowing unit B. Besides, inadequate exchange of air with increased ammonia concentration and relative humidity was observed in farrowing unit A. Outdated technology along with insufficient maintenance of ventilation equipment induces unsuitable microclimatic conditions and increased microbe numbers in the air of farrowing unit, which perhaps is in correlation with an increase in piglet losses. Unlike this, in farrowing units with current housing technology and optimal production results the total microbe numbers are expected to be below 10<sup>4</sup> CFU/m<sup>3</sup> and 10<sup>3</sup> CFU/m<sup>3</sup> of air for bacteria and fungi, respectively.

**Key words:** nursery, piglet losses, microbiological air quality, ammonium

### INTRODUCTION

Air microflora reflects the microbiological condition of the stable and mostly originates from animals and their manure (Methling et al., 1981). It is influenced by airflow and various activities in the facility such as, feed distribution or manure management and dependant on the ventilation system, primary microclimatic conditions, season, and population density (Seedorf, 1998). Therefore, total number of airborne microbes can vary significantly and favor the spreading of disease so the control of animal health status should include the verification of air quality (Bækbo, 1990). In this respect the fattening and farrowing units received most attention in pig production to this date accompanied by comparison of total microbe numbers in the air of these technological phases (Pavičić et al., 2006). However, the aim of this investigation was to compare the microbiological air quality between two farrowing units with different technologies in order to establish possible relationship of airborne microbe concentrations and production results.

## MATERIALS AND METHODS

The investigation was conducted on two different farrowing units during two 28-day production turns. Farrowing unit A with older technology was 270 m<sup>2</sup> in size equipped with 40 pens, while farrowing unit B with contemporary technology was 370 m<sup>2</sup> in size equipped with 60 farrowing pens. Seven air samplings were performed in identical time periods in every production turn with the SAS 100<sup>TM</sup> (PBI International, Italy) air collector on multiple points in the level of animal biozone. Sampling was performed on growth medium for the isolation of mesophylic, hemolytic and coliform bacteria and fungi, which were counted after incubation and expressed per m<sup>3</sup> (CFU/m<sup>3</sup>) of air. Isolated bacteria and fungi were identified by microscope and API tests (Bio Mérieux, France). Control of primary microclimatic conditions: temperature, relative humidity, airflow speed and ammonia concentration was performed alongside every air sampling with the portable digital devices (Testo and Dräger, Germany). The obtained data was subjected to basic statistical analysis with Statistica 7.1 (StatSoft Inc., 2005) software, and eventual significant differences in average bacteria and fungi numbers between two farrowing units were recorded. Microclimatic parameters were calculated as mean values.

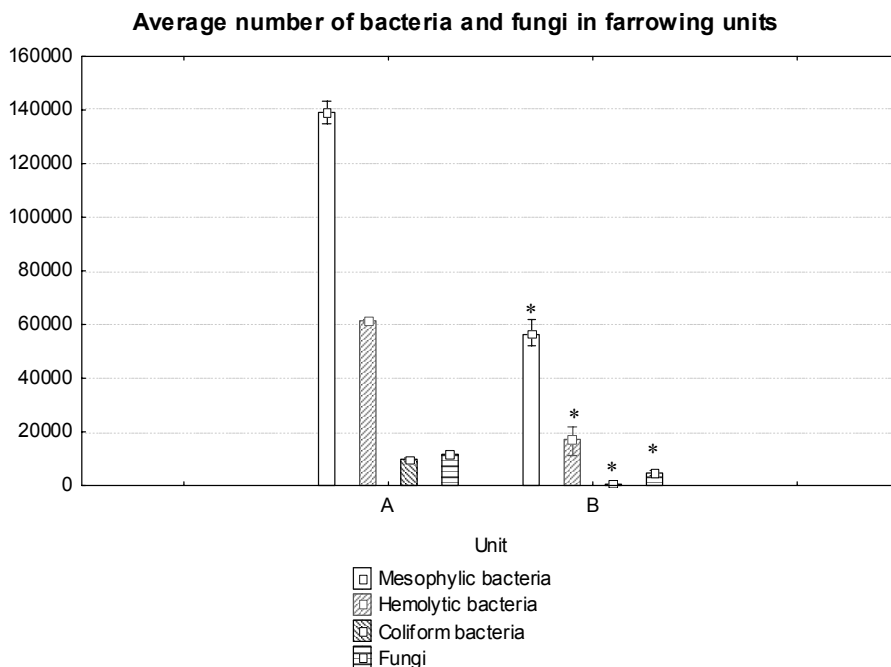
## RESULTS

Average numbers of bacteria and fungi in two different farrowing units during the entire trial period is presented in Table 1 and Graph 1.

**Table 1.** Average number of microbes in the farrowing units during both turns.

Microbes	Farrowing unit with outdated technology (n = 14)		Farrowing unit with modern technology (n = 14)	
	Mean	SD	Mean	SD
Mesophylic bacteria	139678,6	± 2194,110	56357,14*	± 2841,374
Hemolytic bacteria	61228,6	± 1084,456	16992,86*	± 3297,427
Coliform bacteria	9871,4	± 634,191	469,29*	± 45,143
Fungi	11600,0	± 736,938	4650,00*	± 453,618

\* Statistically significant lower value (significance level  $p < 0,01$ ) relative to value recorded in the other facility



**Graph 1.** Average number of bacterial and fungi in the farrowing units during the entire investigation.

\* Statistically significant lower value (significance level  $p < 0, 01$ ) relative to value recorded in the other facility

Gram positive bacteria were predominant in both facilities with 22 bacteria species identified in farrowing unit A and 18 bacteria species identified in farrowing unit B. The proportion of gram negative bacteria was generally lower, but significantly higher in farrowing unit B. Besides, 12 and 8 species of fungi were identified in farrowing units A and B, respectively, whereat *Aspergillus spp.*, and *Trichopyton spp.* were predominant in both facilities. Average microclimatic conditions in farrowing unit A were: temperature  $24^{\circ}\text{C} \pm 1^{\circ}\text{C}$ , relative humidity 82%, airflow speed 0,02 m/s and ammonia concentration 36 ppm. At the same time ammonia values in certain locations in the level of animal biozone were as high as 50 ppm. Average microclimatic conditions in farrowing unit B were: temperature  $26^{\circ}\text{C} \pm 1^{\circ}\text{C}$ , relative humidity 65%, airflow speed 0,2 m/s and ammonia concentration 4 ppm.

## DISCUSSION

Unsuitable zoohygienic conditions could be one of the main factors causing an increase in piglet losses during suckling. At the same time air quality is usually not identified as a cause of the problem so losses can accumulate during prolonged periods of time, raising the question of cost-effectiveness in this phase of pig production. Therefore, increasing number of studies are being

conducted to provide a possible connection between certain health problems and mortality with air quality, thus at the same time searching to find a solution for improving housing conditions for animals including air quality. Modern housing systems with automated microclimate regulation can significantly influence air quality, which is demonstrated by the results of these studies. In fact, according to obtained data it is visible that farrowing unit A, with older and insufficiently maintained technology, had significantly larger numbers of individual microbe species in relation to farrowing unit B. At the same time, gram positive bacteria predominated in both units, from which streptococci and staphylococci constituted more than 80% (Aengst, 1984). Besides, lower values of airflow speed along with high relative humidity and ammonia concentration have been recorded in farrowing unit A. This is an indicator of insufficient ventilation, which can be very hazardous to animal health (Donham, 1995). Such values of primary microclimatic factors can affect an increase in airborne microbe concentration (Attwood et al., 1987), since high relative humidity is favorable in lowering the rate of microbe activity (Jones et al., 1982). It seems that this could be the reason for increased concentration of gram positive bacteria in farrowing unit A that are otherwise present in small numbers as a result of weak survival capability in air (Zucker and Müller, 2002). In so doing, *Enterobacteriaceae* were the predominant species from the family of gram-negative bacteria in the air of farrowing unit A. They will not cause disease by themselves but along with poor microclimatic factors and abundance of gasses they will cause disease and an increase of production losses (Robertson et al., 1990; Hamilton et al., 1998; Andreasen et al., 1999).

## CONCLUSION

The results from farrowing unit A clearly demonstrate the connection between out-dated and insufficiently maintained technology on unacceptable microclimatic conditions, increased numbers of airborne microbes and poor production results in relation to farrowing unit B. Meanwhile in modern farrowing facility while maintaining adequate population density per unit of housing space we can expect the total microbe numbers to be under  $10^4$  CFU/m<sup>3</sup> and  $10^3$  CFU/m<sup>3</sup> of air for bacteria and fungi respectively.

## ACKNOWLEDGMENTS

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## POSSIBILITIES OF KEEPING OPTIMAL PERFORMANCE OF BREEDING BOARS DURING SUMMER PERIOD

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### SUMMARY

Summer is a critical period on pig breeding farms for reproduction. Therefore, in this research effects of a feed supplement in a summer period were studied. Boars were given Ascogen<sup>®</sup> mixed with food during a period of 60 days. The indicators of reproduction successfulness of each boar were collected before the research, after 30 days and finally after 60 days. According to the results, the control and the experimental group were equally successful before the treatment as well as after 30 days of the treatment. After 60 days, however, in the control group conception rate decreased and fewer piglets were born. The experimental group did not have a decline in reproduction.

**Keywords:** boars, summer, reproduction, feed supplement

### INTRODUCTION

Summer is a critical period on pig breeding farms for the reproduction of boars and sows (Love et al., 1993). Environmental factors such as high temperature and long photoperiod can lead to problems both with ejaculate quality and fertilization (Claus et al., 1985; Kunavonkrit et al., 2005).

During hot weather, pigs may eat less (Rinaldo et al., 2000; Nardone et al., 2006) and the nutritional balance of their diet may be affected (Kunavonkrit et al., 2005). In such a way the environment presents a restriction factor for energy intake which animals require in order to carry out specific bodily functions, e.g. to grow and to reproduce, while the continuing failure of the animal to meet its nutrient requirements can be seen as a chronic stressor (Kyriazakis and Savory, 1997; Rinaldo et al., 2000). Furthermore, because of high temperatures, the animal is outside its comfort zone and experiences stress to maintain homeothermy. This requires extra energy in order to thermoregulate, so less energy is available for production processes (Nardone et al., 2006).

In adult boars used for reproduction the decreased nutritional intake has, as a consequence, a decreased libido and a smaller amount of sperm (Levis, 1997). However, some feed supplements may have a positive influence on bodily balance, and, as a consequence, on reproduction. For example, it is believed that feeding a boar with additional vitamin C during stress caused by summer heaths can enhance sperm quality (Lin et al., 1985; Wilson et al., 2004). Similarly, in the researches carried out by Marin-Guzman et al. (1997) boars which were given selenium and vitamin E had better reproduction results.

In spite of difficulties in summer period boars are used for reproduction, which can have a negative effect on their well-being. Therefore in this research, in order to overcome heat stress as

well as potential feed intake problems, boars were given Ascogen<sup>®</sup>. The objectives of this study were to gain optimal reproduction results of breeding boars by applying Ascogen<sup>®</sup> in summer period, giving particular emphasis to safety and welfare of the boars.

## METHODS

The experiment was carried out on a pig breeding farm with 14 mature boars used for semen collecting for AI (artificial insemination).

The research was conducted during the summer months (in June, July and August). The boars were housed individually in pens with outdoor enclosures. Indoor temperatures were between 25°C and 34°C, while outdoor temperatures were between 26°C and 36°C.

Fourteen boars were divided by random choice into a control group (N=7) and an experimental group (N=7). Every day during 60 days the experimental group boars received Ascogen<sup>®</sup> at a concentration of 500 g/ton in a food mixture. Ascogen<sup>®</sup>, powder made of natural substances, is a feed supplement with PSB-Complexes. The main active ingredients of Ascogen<sup>®</sup> are purified RNA (ribonucleic acid), purified nucleotides and precursors, specific organic acids and inactivated yeasts.

The indicators of reproduction successfulness were collected for each boar, i.e. sperm volume (ml) and motility (%), number of inseminated sows, conception rate and return to heat, abortion, as well as the number of live and stillborn piglets two months later. The experimental and the control group data were compared prior to the Ascogen<sup>®</sup> treatment, after 30 days and finally after 60 days of the treatment. Statistical analyses were done with ANOVA ( $p < 0.05$ ).

## RESULTS

Results of the findings in this study are shown in Table 1 and 2 and in Graphs 1, 2 and 3. Statistically relevant differences were found in the control group of boars concerning the sows' conception rate, the number of sows that returned to heat and the number of sows that farrowed. Consequently, smaller number of piglets was obtained from the control group of boars.

**Table 1.** Control group results of reproduction in June, July and August

	Days		
	0	30	60
Sperm volume (ml)*	279 ± 14.87 <sup>a</sup>	286 ± 14.29 <sup>a</sup>	271 ± 14.87 <sup>a</sup>
Sperm motility (%)*	85 ± 4.63 <sup>a</sup>	86 ± 4.68 <sup>a</sup>	82 ± 3.06 <sup>a</sup>
Number of inseminated sows*	6 ± 0.29 <sup>a</sup>	6 ± 0.26 <sup>a</sup>	5 ± 0.34 <sup>a</sup>
Conception rate*	5 ± 0.40 <sup>a</sup>	5 ± 0.40 <sup>a</sup>	3 ± 0.77 <sup>b</sup>
Return to heat*	1 ± 0.20 <sup>a</sup>	1 ± 0.28 <sup>a</sup>	2 ± 0.71 <sup>b</sup>
Abortion*	0 ± 0.00 <sup>a</sup>	0 ± 0.00 <sup>a</sup>	0 ± 0.00 <sup>a</sup>
Number of sows that farrowed*	5 ± 0.40 <sup>a</sup>	5 ± 0.40 <sup>a</sup>	3 ± 0.81 <sup>b</sup>
Number of piglets	55 ± 5.21 <sup>a</sup>	55 ± 5.38 <sup>a</sup>	21 ± 7.04 <sup>b</sup>
Number of live born piglets*	52 ± 4.97 <sup>a</sup>	52 ± 5.21 <sup>a</sup>	20 ± 6.83 <sup>b</sup>
Number of stillborn piglets*	3 ± 0.40 <sup>a</sup>	3 ± 0.52 <sup>a</sup>	0 ± 0.29 <sup>b</sup>

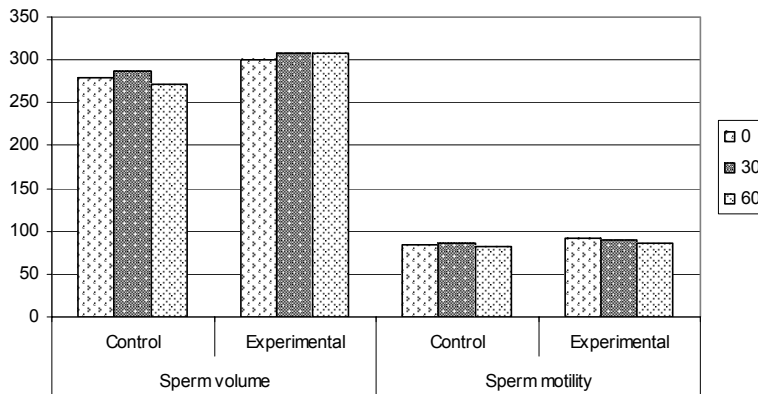
\*Means±S.E. in the same row with different letter differ significantly ( $p < 0.05$ )

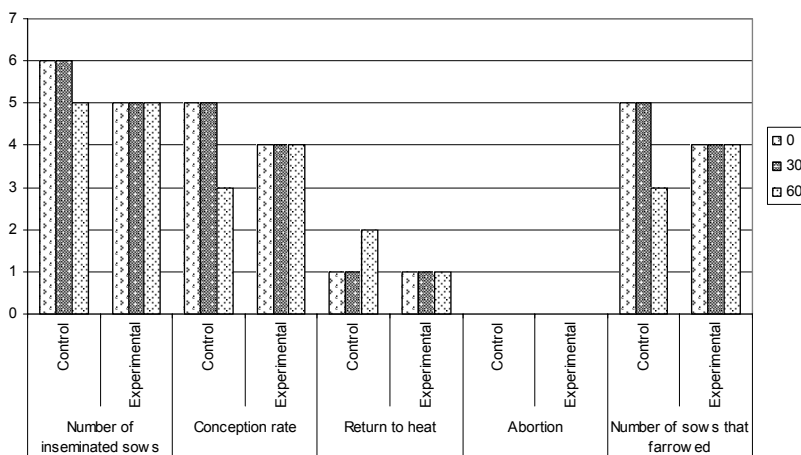


**Table 2.** Experimental group results of reproduction in June, July and August

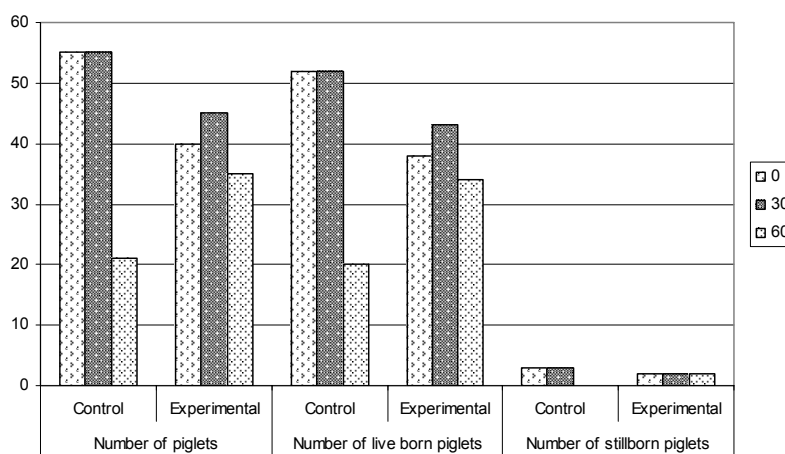
	Days		
	0	30	60
Sperm volume (ml)*	300 ± 34.50 <sup>a</sup>	307 ± 39.98 <sup>a</sup>	307 ± 35.23 <sup>a</sup>
Sperm motility (%)*	91 ± 0.71 <sup>a</sup>	89 ± 1.70 <sup>a</sup>	86 ± 1.80 <sup>a</sup>
Number of inseminated sows*	5 ± 0.69 <sup>a</sup>	5 ± 0.75 <sup>a</sup>	5 ± 0.20 <sup>a</sup>
Conception rate*	4 ± 0.58 <sup>a</sup>	4 ± 0.75 <sup>a</sup>	4 ± 0.47 <sup>a</sup>
Return to heat*	1 ± 0.31 <sup>a</sup>	1 ± 0.44 <sup>a</sup>	1 ± 0.34 <sup>a</sup>
Abortion*	0 ± 0.00 <sup>a</sup>	0 ± 0.00 <sup>a</sup>	0 ± 0.14 <sup>a</sup>
Number of sows that farrowed*	4 ± 0.51 <sup>a</sup>	4 ± 0.71 <sup>a</sup>	4 ± 0.51 <sup>a</sup>
Number of piglets*	40 ± 6.08 <sup>a</sup>	45 ± 9.00 <sup>a</sup>	35 ± 4.87 <sup>a</sup>
Number of live born piglets*	38 ± 5.77 <sup>a</sup>	43 ± 8.55 <sup>a</sup>	34 ± 5.11 <sup>a</sup>
Number of stillborn piglets*	2 ± 0.42 <sup>a</sup>	2 ± 0.53 <sup>a</sup>	2 ± 0.53 <sup>a</sup>

\*Means±S.E. in the same row with different letter differ significantly (p<0.05)

**Graph 1.** Sperm volume and motility in control and experimental group



**Graph 2** Insemination results in control and experimental group



**Graph 3.** Number of piglets born in control and experimental group

### DISCUSSION

Boars used for AI have a great influence on the productivity of the farm (Levis, 1997), and semen production of boars may be influenced by many factors (Park and Yi, 2002). In this research we studied the influence of summer conditions and one feed supplement.

Boars' sperm volume in the control group and in the experimental group ranged, on average, from 271 to 307 ml, while sperm motility was between 82 and 91%. These are common values for breeding boars (e.g. Frangež et al., 2005). According to these indicators of sperm quality, i.e. sperm volume and motility, in every of the three measurements there was no statistically

important difference in sperm quality ( $p>0.05$ ) neither in the control group nor in the experimental group of boars (Graph 1). However, indicators of quality in vitro are not always sufficient indicators for an estimate in vivo (Popwell and Flowers, 2004).

On the other hand, although 5 or 6 sows were inseminated by the sperm of every boar, the conception rate of the control group decreased during the summer period, that is, it fell from 5 successful to 3 successful conceptions (Table 1). The conception rate of the experimental group neither increased nor decreased, and, on average, out of 5 sows inseminated with the sperm of the same boar, 4 became pregnant. On average, in one sow mating did not succeed so she returned to heat (Table 2). As a consequence, the number of sows which farrowed was higher in those inseminated with the sperm from the boars from the experimental group (Graph 2).

Litter size is a complex trait influenced by a paternal, maternal and fetal component and boars have a significant influence on fetal survival rate (Hamann et al., 2004; Popwell and Flowers, 2004). In this research, on average, from one sperm dose from one boar in the control group 55 piglets were born in June and July, whereas in August that number was decreased to 21 piglets, which was significantly less compared to previous months ( $p<0.05$ , Table 1). The experimental group, which was treated with Ascogen<sup>®</sup> for 60 days, did not have a decline in reproduction during three summer months (Table 2, Graph 3).

## CONCLUSION

Environmental conditions during summer are unfavourable for boars to maintain homeostasis and reproduction but it is possible to improve it with feed supplement. Therefore, it is recommended to treat breeding boars with Ascogen<sup>®</sup> as a measure of prevention of summer losses.

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## EFFECTIVENESS OF A NEW BIOCIDES AGENT IN ANIMAL PRODUCTION

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### SUMMARY

The oxygen emitting agents are used frequently in general disinfection owing to insignificant corrosion effect. In contribution are present results of disinfection of pig stables with a new biocide agent Virucidal Extra in practical conditions. Study was performed on the typical large pig farm in Slovenia in piglet and fattening pig stables and six smaller common pig farms in real field conditions following typical sanitation routine by farm staff. Reduction in number of CFU on tested surfaces was by disinfection in average range between 74 and 94%.

**Keywords:** disinfection, biocide, peroximonosulfate, prevention, pig houses, Virucidal Extra

### INTRODUCTION

Effectiveness of sanitation process is influenced by several factors as disinfectant agents, temperature, exposition time and presence of organic matter. The oxygen emitting agents are used frequently in general disinfection owing to insignificant corrosion effect. In contribution are present results of disinfection of pig stables with a new biocide agent Virucidal Extra in practical conditions.

Virucidal Extra® is a well-balanced, stable mixture of inorganic peroxide compound, inorganic salts, halogen group donor, anionic surface-active substance, colouring agent and lemon flavour. It rapidly and effectively kills all known viruses, Gram-positive and Gram-negative bacteria, and fungi. It acts also when highly diluted. Due to its broad biocide action, it is also suitable for:

- stables of all types
- transportation vehicles
- veterinary facilities (stations, laboratories, surgeries etc.)
- areas for housing of animals (livestock buildings, dog kennels, cages...)
- areas for preparation, processing and storage of food and feed/feedstuffs
- hatcheries
- fish stables
- disinfection barriers for vehicles and footwear

## MATERIAL AND METHODS

Study was performed on the typical large pig farm in Slovenia in piglet and fattening pig stables and six smaller common pig farms in real field conditions following typical sanitation routine by farm staff. The efficiency of biocide agent (BA) was estimated by reduction of the number of colony forming units (CFU) on different surfaces in stables. For final disinfection 1% working solution was used in amount of 300 ml/m<sup>2</sup>. The total surface expense for disinfection in each stable was defined by multiplying the floor surface by 2.5.

The number of CFU was detected by smears following parts of surfaces: corridor (feed passage, walking corridor and pier), feeder, cubicle floor (slatted, solid floor), wall, cubicle fence, and fan and water nipple. Smears were prepared in a standard laboratory condition for CFU on 30°C for 72 hours.

CFU on surfaces were assessed before cleaning, after cleaning and drying and after disinfection. On each pig stable 41 smears in 3 repetitions were taken. In total 1.599 smears were taken. Simultaneously measurements of the microclimate parameters (air temperature, relative humidity, air speed), surface temperature, surface pH, hardness of water, estimated water spots remaining, dust and annotation of implemented sanitation routine (cleaning, disinfection) in tested stables were performed.

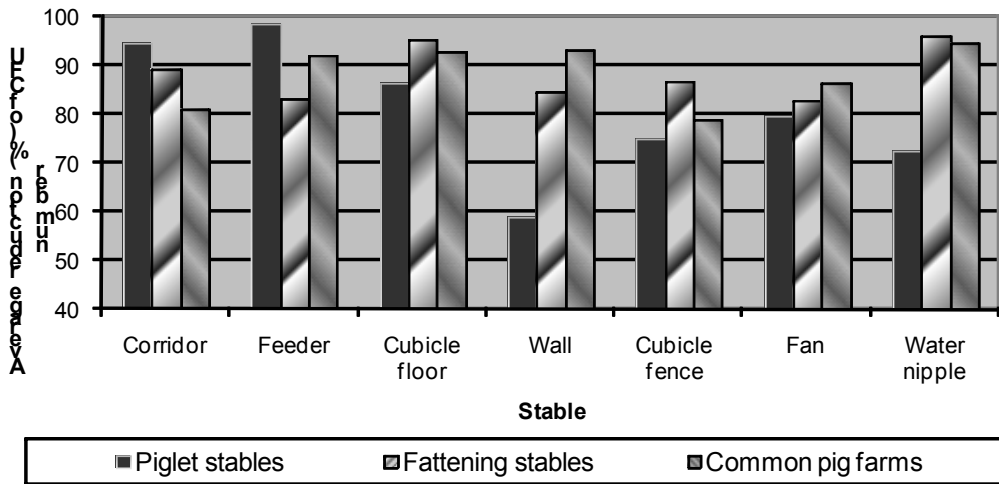
Results of measurements were tabled and statistically evaluated.

## RESULTS

Results are present in a table 1 and figure 1. After disinfection reduction in number of CFU on tested surfaces varied in average range between 74 and 94%. Reduction in number of CFU on tested surfaces: corridor (feed passage, walking corridor and pier) 81.0–94.7%, feeder 83.1–98.4%, cubicle floor 86.4–95.3% (slatted, solid floor), wall 59.1 and 93.2%, cubicle fence 75.0, fan 79.6–86.5% and water nipple 72.6–96.0%.

**Table 1.** Average reduction (%) of CFU number in pig stables on different tasted surfaces

Stable	Piglet stables	Fattening pig stables	Common pig farms
<b>Corridor</b> (feed passage, walking corridor, pier)	94,7	89,3	81,1
<b>Feeder</b>	98,4	83,1	92,2
<b>Cubicle floor</b> (slatted, solid floor)	86,4	95,3	93,0
<b>Wall</b>	59,1	84,8	93,2
<b>Cubicle fence</b>	75	86,8	78,9
<b>Fan</b>	79,6	82,7	86,5
<b>Water nipple</b>	72,6	96,0	94,8



**Figure 1.** Average reduction (%) of CFU number in pig stables on different tasted surfaces

## CONCLUSIONS

Biocidal agent Virucidal Extra is an effective agent in general disinfection of stables in animal production. Effectiveness was found out also in pure cleaning routine.

Virucidal in this clinical trial represent very quality and in some cases an exceptional disinfectant action. Virucidal was especially successful in dirtiness aggravated environment. All findings are average results of sample smearing results from different types of breeding and animal categories in pig stabling referring especially to the most aggravated and exposed places.

Many deficiencies in sanitary technologies of pig stables were found; especially in the sense of none continually process of animal emptying, cleaning and disinfection. Few days delay among animal emptying and stable cleaning have the consequence in a high increase of micro-organism in dirty medium, what aggravate also Virucidal efficiency, but never the less the Virucidal was successful on those conditions as well. Furthermore it was established that cleaning and disinfection process in fattening pig stables done by high-pressure machines, because bacteria aggravated aerosol, which can be potentially dangerous for additional surface contamination also after finished disinfection. Workers, who perform cleaning and disinfection of stable and equipment, do not handle all surfaces equally, so we establish remains of unclean and un-disinfected places especially on equipment with heavier access or on surfaces which were not directly exposed. Exactly on those places Virucidal had successful disinfection effects on the number of CFU, what have very practical meaning. In common pig stables the same phenomenon appeared. Cleaning and disinfection in large farm was more successful as in common pig stables, while breeders were not splashing surfaces enough after cleaning what can cause microbiological recontamination of surfaces that can be in some cases higher after cleaning as before it. Mechanical cleaning and use of high-pressure machines, regarding to this clinical trial, were the reason for microbiological contaminated aerosol what can recontaminate surfaces after sedimentation.

If we compare results of CFU reduction in piglet and fattening stables after cleaning process we can establish that in fattening pig stables CFU reduction was worse or even negative (more CFU after cleaning). Moreover it is astonishing that Virucidal action was better in fattening pig stables despite worse conditions for disinfectant activity. Virucidal reduction of CFU varies between 74 and 94%, and between 79 and 97% in fattening pig stables. In analysis due to criteria only, the reduction of tested surfaces was between 72 and 98%, and between 83 and 96% in fattening pig stables, except in one case with higher CFU after disinfection as before it (fan activity). In common pig stables we established poor Virucidal activity as in previous stables, encountered because of poor cleaning. It should be emphasized that Virucidal efficiency is still 81 to 93% despite very poor cleaning results. Obviously very heterogeneous CFU pattern was represented due to unequally cleaned surfaces and equipment in common pig stables had important influence on Virucidal activity.

Virucidal test have been done in summer weather by temperatures between 19 and 32°C. Temperatures of disinfected surfaces were between 18 and 23°C. Temperature differences from surfaces of sample smearing were too small for influencing to results. Hardness of water was very similar in all stables – between 8.2 and 8.7 dH, as well as surface pH, which were in alkaline line between 8.2 and 8.7. Humidity and air speed were also in allowed areas of normative.

From all it can be concluded that disinfectant Virucidal extra demonstrated qualitative disinfection effect in practical criteria. Excellent preparation activity represented also in dirty environment, where most of other disinfections lost their efficiency. For that reason Virucidal extra is by our opinion an appropriate choice for disinfection in pig stables. Along that we appeal on improving cleaning and disinfection technologies which can be of crucial for Virucidal efficiency.

For further assessing of disinfecting effectiveness of agent are recommended studies of specific effects on separate pathogenically micro-organisms.





**A3 –  
IMPACT OF ENVIRONMENT ON POULTRY  
BEHAVIOUR, HEALTH AND PERFORMANCE**



## ORAL PRESENTATIONS

### BIOAEROSOL IN LAYING HEN HOUSE

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#### ABSTRACT

Intensive production and housing of laying hens result in a significant amount of hazardous pollutants in the air of poultry house. Under specific conditions, these pollutants can affect the health of both poultry and people who work in poultry houses. The study was carried out in winter period on a farm with a capacity of 17000 Shaver hybrid laying hens from 25<sup>th</sup> week of production. Laying hens were housed in cages, 8–10 *per* cage. Samples were collected in the morning once a week for six weeks, at 5 sites in the house. Air was sampled by use of a Merck MAS-100 (Merck KgaA, Darmstadt, Germany) device onto commercial nutrient and Sabouraud agar (Biolife, Milan, Italy). Upon incubation, microorganisms grown on the medium (bacteria and fungi) were counted and predominant species were inoculated for determination. Dust was sampled by an SKC pump (SKC Ltd., Blandford Forum, UK) on filters (Whatman International Ltd., Maidstone, UK). Temperature (t °C), relative humidity (rh %) and air velocity (w m/s) were determined by a Testo 400 (Testo Inc., Lenzkirch, Germany) device. The concentration of ammonia and carbon dioxide was determined by a Dräger-Multiwarn II (Dräger, Darmstadt, Germany) device. The measured values of study parameters were processed by Microsoft Excel and Statistica 6 software. Descriptive statistics was employed and statistical significance at 5% (p<0.05) was determined by Student's t-test. The concentration of bacteria ranged from 1.6 x 10<sup>2</sup> to 2.7 x 10<sup>3</sup> cfu/m<sup>3</sup>, of fungi from 0.8 x 10<sup>2</sup> to 6.9 x 10<sup>2</sup> cfu/m<sup>3</sup>, and of dust from 1.6 to 3.8 mg/m<sup>3</sup>. The mean level of ammonia was between 5.87 and 9.22 ppm. The predominant bacteria were from the genera *Staphylococcus* and *Streptococcus*, and fungi from the genera *Aspergillus* and *Penicillium*. The results on all microclimate parameters were in line with recommended standards. The low air count of the bacteria, fungi and dust could be attributed to the relatively low temperature recorded in the housing and its environment.

#### INTRODUCTION

Good hygiene of housing air is a major prerequisite for poultry health and productivity. In addition, poor quality of air in poultry housing can have adverse effects on the health of people working there (Stetzenbach et al., 2004). Intensive poultry production is known to be a source of numerous air pollutants including microorganisms, dust, gases, endotoxins, and offensive odor

(Takai et al., 1998; Zang, 1999). This form of contamination can be caused by inappropriate zoohygienic conditions in the housing due to inadequate or poor ventilation, overcrowding, etc. All particles present in the animal housing air, which contain microorganisms, desquamated epithelium, dried feces and other organic particles, are known under the common term of bioaerosol. In addition to the components mentioned above, bioaerosol can contain live and dead bacteria, parts of fungi, spores, mycotoxins and tannins. Bioaerosol concentrations found in the animal housing air vary depending on the animal keeping and housing conditions, age, method of feeding and feces/urine disposal, etc. Generally, air hygiene frequently presents an unsatisfactory and limiting factor of poultry productivity, health and welfare.

### MATERIAL AND METHODS

The study was conducted during winter period at a farm with a capacity of 17000 Shaver hybrid laying hens from 25<sup>th</sup> week of production. Laying hens were housed in cages, 8–10 *per* cage. Samples were collected in the morning once a week for six weeks, at 5 sites in the house. Air was sampled by use of a Merck MAS-100 (Merck KgaA, Darmstadt, Germany) device onto commercial nutrient and Sabouraud agar (Biolife, Milan, Italy). Upon incubation, microorganisms grown on the medium (bacteria and fungi) were counted and predominant species were inoculated for identification. Dust was sampled by an SKC pump (SKC Ltd., Blandford Forum, UK) on filters (Whatman International Ltd., Maidstone, UK). Temperature ( $t$  °C), relative humidity (rh %) and air velocity ( $w$  m/s) were determined by a Testo 400 (Testo Inc., Lenzkirch, Germany) device. The concentration of ammonia and carbon dioxide was determined by a Dräger-Multiwarn II (Dräger, Darmstadt, Germany) device. The measured values of study parameters were processed by Microsoft Excel and Statistica 6 software. Descriptive statistics was employed and statistical significance at 5% ( $p < 0.05$ ) was determined by Student's t-test (Anonymous, 1994; Petz, 2001).

### RESULTS AND DISCUSSION

Elevated bioaerosol concentration in poultry housing occurs consequentially to animal accommodation conditions (high population density, dry litter) and technology process (various manipulations). In such a setting, the air is the source and storage of various microorganisms, mostly originating from animals (80%) and their droppings. In the overall microorganism count, the genera *Staphylococcus* and *Streptococcus* account for 60% and 30%, respectively, the rest being fungi, spores and other microorganisms, however, the majority of animal housing microflora is nonpathogenic (Hartung, 1994). Many authors report on the varying bioaerosol concentration in the air of animal housing, being highest in poultry housings irrespective of poultry keeping on thick litter or in cages (Wathes, 1994; Radon et al., 2002). Otherwise, the concentration of bioaerosol depends on the number of animals, animal population density *per* area unit, type and quality of litter, ventilation, etc. (Matković et al., 2006).

Concerning gaseous air pollutants, mention should be made of ammonia produced by fecal nitrogenous organic substance decay, and of carbon dioxide. Poor ventilation of animal housing results in elevated concentrations of ammonia and carbon dioxide, which have adverse effects on the poultry health and productivity. According to Hartung (2005), the maximal allowed concentration in the air of poultry housing is 20 ppm for ammonia, 3000 ppm for carbon dioxide,

10 ppm for hydrogen sulfide, and 50 ppm for carbon monoxide. Poultry have a considerably lower tolerance to ammonia than other animals, so a concentration of 20 ppm causes irritation of the mucous membranes of the eyes and respiratory system, reduced feed intake, and occurrence of technological runts (Kristensen and Wathes, 2000).

The results obtained in the present study indicated the level of environmental air contamination with bioaerosol to be consistent with literature data, approaching the lower limit reported (Hartung, 1994; Seedorf et al., 1998; Radon et al., 2002; Hyvärinen et al., 2006). The concentration of bacteria ranged from  $1.6 \times 10^2$  to  $2.7 \times 10^3$  cfu/m<sup>3</sup> air, predominated by the genera *Staphylococcus* sp. and *Streptococcus* sp., *Escherichia coli*, *Pseudomonas* sp., *Klebsiella* sp. and *Micrococcus* sp (Table 1 and 2). The concentration of fungi ranged from  $0.8 \times 10^2$  to  $6.9 \times 10^2$  cfu/m<sup>3</sup> air, predominated by the genera *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp (Table 1 and 2). The concentration of dust during the six production weeks ranged from 1.6 to 3.8 mg/m<sup>3</sup> air (Table 1 and 2). The mean level of ammonia was between 5.87 and 9.22 ppm. The low air concentration of the microorganisms and dust could be attributed to the relatively low temperature during the study period (winter) recorded in the housing and its environment, generally characterized by lower animal activity. A higher bioaerosol concentration was only recorded in the sixth week of the study, when the values of air temperature, relative humidity and ammonia showed a slight increase. A significant differentiation in the bacterial, fungi and dust concentration was recorded between all observed weeks as demonstrated by t-test yielding statistical significance at a level of  $p < 0.05$  (Table 3).

Other microclimate indicators were generally within the allowed limits. Relative humidity in the poultry house ranged between 40% and 70%, as recommended (Whyte, 1993). Increased dust concentration may be associated with lower humidity, which has adverse effects on the poultry respiratory system.

**Table 1.** Mean levels of total bacterial count, fungi count, dust concentration and microclimate parameters in laying hen housing air

Parameter	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
<b>bacteria</b> cfu/m <sup>3</sup>	$1,6 \times 10^2$	$1,9 \times 10^2$	$1,2 \times 10^3$	$5,7 \times 10^2$	$1,1 \times 10^3$	$2,7 \times 10^3$
<b>fungi</b> cfu/m <sup>3</sup>	$0,8 \times 10^2$	$3,5 \times 10^2$	$2,8 \times 10^2$	$2,3 \times 10^2$	$6,9 \times 10^2$	$5,4 \times 10^2$
<b>dust</b> mg/m <sup>3</sup>	1,6	2,3	3,8	2,9	3,1	2,2
<b>temp.</b> °C	15,86	16,84	15,76	16,23	16,59	17,89
<b>humid.</b> %	66,04	59,92	63,29	62,20	62,19	69,55
<b>airflow</b> m/s	0,07	0,13	0,08	0,08	0,09	0,06
<b>NH<sub>3</sub></b> ppm	5.87	6,11	6,22	8,67	7,99	9,22
<b>CO<sub>2</sub></b> %	0,08	0,09	0,12	0,07	0,11	0,15

**Table 2.** Descriptive statistical analysis of bacteria, fungi, dust and microclimate factors recorded in laying hen housing air

	Week	n	arithmetic mean	minimum	maksimum	variance	SD	SE
bacteria cfu/m <sup>3</sup>	1	5	1,60 x 10 <sup>2</sup>	1,60 x 10 <sup>2</sup>	1,60 x 10 <sup>2</sup>	0,01	0,09	0,04
	2	5	1,90 x 10 <sup>2</sup>	1,90 x 10 <sup>2</sup>	1,90 x 10 <sup>2</sup>	0,00	0,01	0,00
	3	5	1,20 x 10 <sup>3</sup>	1,20 x 10 <sup>3</sup>	1,20 x 10 <sup>3</sup>	0,00	0,01	0,01
	4	5	5,70 x 10 <sup>2</sup>	5,70 x 10 <sup>2</sup>	5,70 x 10 <sup>2</sup>	0,00	0,01	0,00
	5	5	1,10 x 10 <sup>3</sup>	1,10 x 10 <sup>3</sup>	1,10 x 10 <sup>3</sup>	0,00	0,01	0,00
	6	5	2,70 x 10 <sup>3</sup>	2,70 x 10 <sup>3</sup>	2,70 x 10 <sup>3</sup>	0,00	0,01	0,00
fungi cfu/m <sup>3</sup>	1	5	8,00 x 10	8,00 x 10	8,00 x 10	0,00	0,01	0,01
	2	5	3,50 x 10 <sup>2</sup>	3,50 x 10 <sup>2</sup>	3,50 x 10 <sup>2</sup>	0,00	0,01	0,01
	3	5	2,80 x 10 <sup>2</sup>	2,80 x 10 <sup>2</sup>	2,80 x 10 <sup>2</sup>	0,00	0,01	0,01
	4	5	2,30 x 10 <sup>2</sup>	2,30 x 10 <sup>2</sup>	2,30 x 10 <sup>2</sup>	0,00	0,00	0,00
	5	5	6,90 x 10 <sup>2</sup>	6,90 x 10 <sup>2</sup>	6,90 x 10 <sup>2</sup>	0,00	0,01	0,00
	6	5	5,40 x 10 <sup>2</sup>	5,40 x 10 <sup>2</sup>	5,40 x 10 <sup>2</sup>	0,00	0,00	0,00
dust mg/m <sup>3</sup>	1	5	1,60	1,60	1,60	0,00	0,00	0,00
	2	5	2,30	2,30	2,30	0,00	0,00	0,00
	3	5	3,80	3,80	3,80	0,00	0,00	0,00
	4	5	2,90	2,90	2,90	0,00	0,00	0,00
	5	5	3,10	3,10	3,10	0,00	0,00	0,00
	6	5	2,20	2,20	2,20	0,00	0,00	0,00
microclimate	temp °C	5	16,53	15,76	17,89	0,53	0,73	0,13
	humid. %	5	63,87	59,92	69,55	10,09	3,18	0,58
	airflow m/s	5	0,09	0,06	0,13	0,00	0,02	0,00
	NH <sub>3</sub> ppm	5	7,35	5,87	9,22	1,84	1,36	0,25
	CO <sub>2</sub> %	5	0,10	0,07	0,15	0,00	0,03	0,00

**Table 3.** t-test for dependent variables at p<0.05

Parameter		n	t	p
bacteria cfu/m <sup>3</sup>	1 week – 2 week	5	-7,40E+02	0,00
	2 week – 3 week	5	-1,52E+05	0,00
	3 week – 4 week	5	1,68E+05	0,01
	4 week – 5 week	5	-1,68E+05	0,01
	5 week – 6 week	5	-2,26E+05	0,01
fungi cfu/m <sup>3</sup>	1 week – 2 week	5	-1,10E+05	0,00
	2 week – 3 week	5	1,11E+04	0,00
	3 week – 4 week	5	8,33E+03	0,00
	4 week – 5 week	5	-1,15E+06	0,01
	5 week – 6 week	5	6,82E+04	0,00
dust mg/m <sup>3</sup>	1 week – 2 week	5	-8,14E+15	0,00
	2 week – 3 week	5	-3,06E+03	0,01
	3 week – 4 week	5	1,84E+03	0,00
	4 week – 5 week	5	-6,32E+02	0,00
	5 week – 6 week	5	2,85E+03	0,00

## CONCLUSION

In the air of housing for laying hens determined concentration of bioaerosols was in the lowest limits known from literature. Within observed six weeks of production exist significant differentiation in bioaerosol concentration that toward the end of research have significant increase.

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## CONTROLLING THE CONCENTRATIONS OF AIRBORNE POLLUTANTS IN POULTRY BUILDINGS

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### SUMMARY

The objective of the project was to assess the efficiency of an oil treatment applied to the bedding material in broiler buildings in order to improve air quality. A classical comparative experiment was conducted during the winter season on a commercial farm. Bedding in a broiler building was treated with a mixture of oil/water, while the other identical building was used as control. Air quality parameters were measured in the building using standard measuring techniques. The oil treatment significantly ( $p < 0.001$ ) reduced the concentrations of both inhalable and respirable particles in the treatment room. However, the mortality rate in the oil treated building was 6.59% compared to the control room of 3.83%, which would require further investigation.

**Keywords:** poultry, air quality, spraying, reduction, emission, ammonia, dust

### INTRODUCTION

The present economic climate of poultry production forces producers to focus on improving efficiency. One of the important factors in achieving improved efficiency is the provision of an optimal building environment (Backstrom *et al.* 1994). Optimal environment encompasses good air quality including gas, particles and microbial concentrations as well as controlled temperature, humidity and ventilation rates (Wathes *et al.* 1991; Wathes *et al.* 1983). An improvement in air quality within poultry buildings should enhance production efficiency and health of birds (Almond *et al.* 1996) as well as reduce OH&S related health problems in humans (Donham *et al.* 1989). The litter is a major source of particles in poultry houses and its characteristics would affect airborne particle concentrations. Therefore, the most likely factors, which can be controlled to achieve a reduction in the concentration of airborne particles in poultry buildings, are the quality and characteristics of the bedding material. The aim of the project presented here was to conduct experiments to assess a strategy aimed at improving air quality in poultry buildings and therefore the sustainability of the farming operation. It was hypothesised that dramatic airborne particle reduction could be achieved by sprinkling oil directly onto the litter. It was expected that this treatment would modify the properties of the litter, making smaller, potentially airborne particles stick to the large bedding particles and therefore reduce the opportunities airborne particle generation (Drost *et al.* 1999).

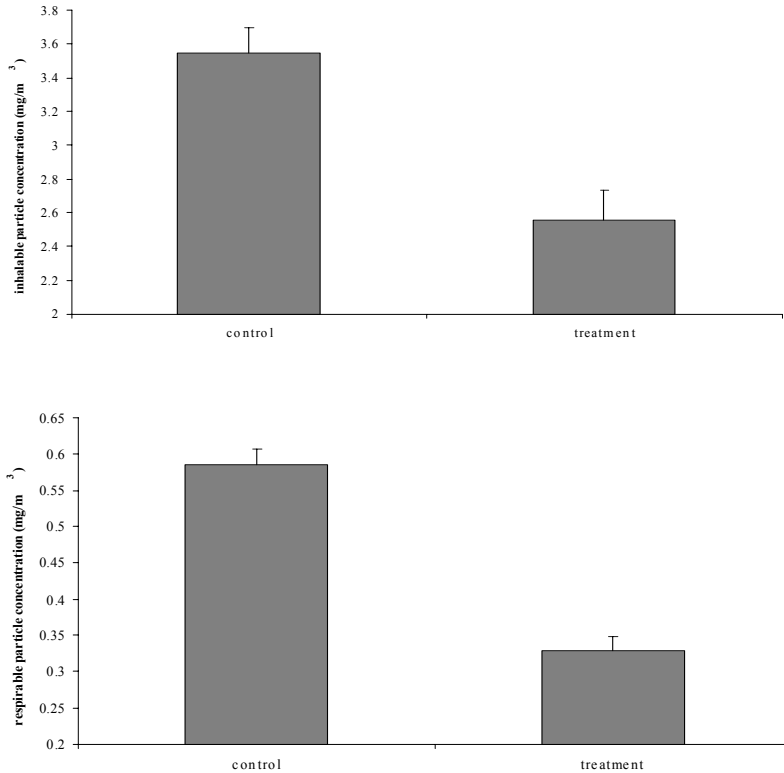
## MATERIAL AND METHODS

A classical comparative experiment was conducted during the winter season in 2002 in South Australia. Two identical and environmentally controlled broiler buildings (approximate size of 370 m<sup>2</sup>) on the same poultry farm were selected for the experiment and chopped straw was used as litter material in both buildings. The bedding material in one of the buildings was treated with the oil/water mixture, while the other building stocked at the same rate was used as control building. Male meat birds were placed in the buildings at the same time and were stocked at 15.9 birds/m<sup>2</sup>. The light program was a continuous ten hours dark period from 5 p.m. to 3 a.m. The incorporation rate of oil was based on the results of the preliminary shaker-box trial (Banhazi *et al.* 2002). The quantity of oil used represented approximately 7.5% of the weight of the bedding material. The treatment was applied after the chopped straw was spread inside the buildings and before the birds were introduced. Canola oil, water and surfactant (emulsifier) were mixed in a drum at the ratio of 8:4:1. The water was incorporated at a minimum rate to prevent excessive wetting of the litter, however some water was necessary to facilitate spraying of the mixture. The high viscosity of the oil made it difficult to use any low-pressure spraying instrumentation for the delivery of the oil treatment. The mix was poured into a backpack-spraying unit containing 16 litres of the mixture and sprayed directly onto the litter that was then raked to homogeneously spread the mix.

The day after the oil treatment, wire cages were positioned in the middle of both buildings to protect the measuring devices, which were deployed within these cages in both the buildings. Temperature and humidity sensors were used to collect both internal and external humidity/temperature data. The inhalable and respirable particles concentration inside the buildings was measured twice a week, for the duration of the study utilising the standard gravimetric method (Banhazi *et al.* 2004). The dust pumps were operated over a 6-hour period, using programmable timers. The sampling periods were set from 10 am to 4 pm every Tuesday and Thursday to coincide with the highest level of activity expected in the buildings. Ammonia and carbon dioxide were monitored continuously using a multi-gas monitoring machine in each building (Banhazi *et al.* 2005). The producer recorded mortality per building every day. A General linear Model (GLM) (Statistica 6.0) was developed to determine the effects of the oil treatment on inhalable and respirable airborne particles concentrations, considering age of birds and other environmental covariates such as internal humidity and temperature. The GLM developed to determine the effect of oil treatment on ammonia concentration incorporated, internal humidity, internal temperature, bedding temperature and CO<sub>2</sub> concentrations as covariates.

## RESULTS AND DISCUSSION

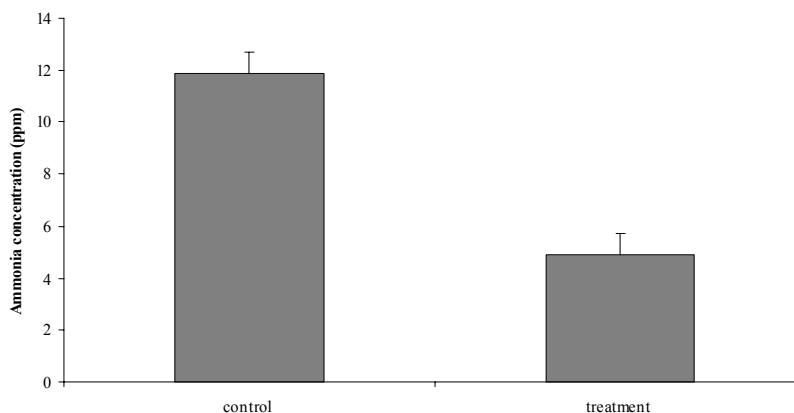
Figures 1 show the mean concentrations of inhalable and respirable airborne particles in the treatment and control rooms. The oil treatment significantly ( $p < 0.001$ ) reduced the concentrations of both inhalable and respirable particles in the treatment room, which confirmed the effect of oil treatment demonstrated in previous studies (Banhazi *et al.* 1999; Feddes *et al.* 1995).



**Figure 1.** The LS mean ( $\pm$  SE) inhalable and respirable particle concentrations in control and trial buildings

The age of birds also had a significant effect on inhalable particles concentration ( $p < 0.05$ ) but not on respirable particles. The inhalable particles concentration increased with the age of birds, which agreed with previous studies (Hinz & Linke 1998; Madelin & Wathes 1989; Renault 1997). The internal temperature significantly ( $p < 0.05$ ) affected the respirable particles concentration although it was not statistically significant for the inhalable particles concentration.

A significant reduction in ammonia concentration ( $P < 0.001$ ) was also demonstrated in the treatment room. Figure 2 shows the mean ammonia concentration in control and trial rooms. A reduction of ammonia concentrations with the same type of oil treatment was reported in piggery buildings (Zhang 1997). The effect of the oil treatment on ammonia was not demonstrated in previous studies in poultry buildings (Feddes et al. 1995). One possible explanation for the positive effect demonstrated in this study is that the oil treatment might interfere with the bacteria flora in bedding responsible for ammonia generation from nitrogenous compounds, thus decreasing the ability of bacteria to generate ammonia. Despite the fact that the reduction of ammonia concentrations has not been fully explained, this finding was an important result because high ammonia levels are not advantageous for poultry production.



**Figure 2.** LS Mean ( $\pm$  SE) ammonia concentrations in control and treatment buildings

### Practical aspects and mortality

This application has to be made more practical via associated engineering developments, as the experimentally used manual spraying and raking is not practical under commercial conditions. In the future the oil can be directly incorporated into the bedding material during the processing of the straw. It would be easy to set up a small sprayer machine on the conveyor belt that carries the chopped straw inside the building. According to the collaborating producer, the reduced airborne particle generation from the bedding material during the spreading of the straw was another beneficial aspect of the trial. The spreading of the bedding material is normally associated with high airborne particle concentrations and the reduction of airborne particles during that time most likely had a major beneficial effect on worker safety and respiratory health.

After the application of the oil mixture, casual observation indicated that birds were as active scratching the bedding in the control building as in the experimental building. Therefore, the oil treatment appeared not affect the comfort characteristics of the bedding for the young birds. Moreover the plumage of the birds was not greasy. Greasy plumage could cause heat loss in birds reported in other studies (McGovern *et al.* 2000). The overall cost associated with the treatment of the trial building (80 litres of oil and 10 litres of surfactant applied) was \$166 or AU\$ 0.45/m<sup>2</sup>. The mortality rate in the oil treated building was 6.59% compared to the control room of 3.83%. In previous studies with the application of oil treatment to the litter in poultry buildings, no adverse effect on mortality was demonstrated (Feddes *et al.* 1995; McGovern *et al.* 1999). However, the increased mortality observed in this study, warrant further investigation.

Overall, the study conducted have demonstrated that airborne particle and ammonia concentration can be significantly reduced in broiler buildings (using straw bedding) by impregnating the litter with a relatively small amount of canola oil, once before the birds enter the building (Banhazi *et al.*, 2003). The demonstrated (and unexpected) significant reduction in ammonia indicated, that this technology could potentially be used to reduce ammonia emissions as well.

## CONCLUSION AND IMPLICATIONS

The high particle concentrations found in poultry buildings motivated researchers to experiment with this particle reduction technique under Australian climatic conditions. The application of an oil/water/emulsifier mixture directly onto the litter at the beginning of a batch of broilers; (1) significantly reduced the concentrations of inhalable and respirable airborne particles and (2) ammonia. However, the apparent higher mortality in the treatment group is noteworthy and may warrant further investigation.

This particle reduction technique demonstrated its efficiency in terms of improving the indoor air quality and it can be also assumed the occupational health and safety (OH&S) conditions were better for the producers as a result of the treatment. In addition, the reduction of particle levels indoors will also reduce particle emissions. The future adoption of particle reduction strategies in the intensive livestock production industry and particularly in poultry buildings is important, due to the increasing environmental and occupational health and safety requirements. The oil impregnation method, utilised during this experiment, appears to be useful and practical. However, further experiments will be needed to assess the potentially beneficial effects of particle reduction on production efficiency, which will encourage poultry producers to apply this technique.

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## EFFECTS OF THE INTRODUCTION OF LACTIC ACID BACTERIA IN THE FEED OF BROILERS: FROM THE FARM TO THE PROCESSING PLANT

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### SUMMARY

This trial aims to assess the effect of a feed complement (*Lactobacillus* spp.) in broilers in order to find an alternative to the antibiotic growth promoters. The trial was performed in the AFSSA experimental husbandry where the animal performances were recorded regularly, up to the slaughterhouse. At the rearing level, sampling included faecal content, while at the processing level, samples from neck and thigh skins were taken. Coliforms, *C. perfringens*, *Enterococcus*, *S. aureus*, *Pseudomonas* and Lactic acid bacteria were enumerated in these samples. The results showed a significant improvement in daily weight gain (P=0,049) in the batch receiving the lactic acid strain at 10<sup>5</sup> cfu/ml. At this concentration, the product showed a positive effect (P=0,031) on the health of the birds. Regarding the digestive flora, no significant effect was recorded in the samples. The product did not modify the bacterial count of the treated batches nor at the rearing neither at the processing level.

**Keywords:** alternative to AGP, digestive flora, lactic acid bacteria, poultry production

### INTRODUCTION

The ban of antimicrobial growth promoters (AGP) from poultry feed has led to a decline in animal health status, an arise in digestive troubles (Puterflam et al., 2007) and the use of therapeutic drugs (Grave et al., 2004). In turn, this could affect the colonisation of the animal intestinal tract by opportunistic bacteria and/or pathogens, leading to a degradation of the hygienic quality of animal products meant for human consumption. Moreover, the microbiological quality of processed poultry meat is directly linked to the flock contamination via the slaughtering process and the contamination of carcasses mainly during evisceration. In study aiming to assess the risk of *Salmonella* contamination of turkey carcasses, it was shown that the risk of meat contamination at the slaughterhouse and cutting plant is associated with the carriage rate in live animals: a group with a low carriage rate represents a moderate risk (0 to 4% contaminated meat), while one with a high carriage rate represents a higher risk (11 to 40% contaminated meat) (Petton et al., 2003). There is therefore an increasing need to find alternatives to the use of AGP efficient in reducing the risk of animal diseases, improving the balance of the gut microflora and allowing reducing pathogen shedding. In this regard, a previous study aimed to screen different “natural” products led to the selection of a lactic acid bacteria based product regarding its efficiency to reduce the shedding of *Salmonella* by young turkeys (Petton et al., 2005).

The aim of this study is to test the effect of this lactic acid bacteria based product regarding the growth performances and the digestive flora equilibrium balance during the rearing and the influence on the microbiological quality of the carcasses at the processing level.

## MATERIAL AND METHODS

### *Animals*

5000 "Ross" broilers were divided into 15 batches distributed over 3 experimental treatments. Each treatment was replicated five times with 250 animals in each floor pen leading to a stocking density of 16 animals/m<sup>2</sup>.

### *Lactic complement*

The lyophilised lactic acid bacterial strain was added to the drinking water. The birds of the treatment 1 (T1) received 10<sup>5</sup> cfu/ml, and those of the treatment 2 (T2) received 10<sup>6</sup> cfu/ml, while in the control treatment (T0); the animals did not receive the product.

### *Animal performances*

Animal growth performances were recorded by weighing a sample of 30 animals by floor pen at 7, 21 and 36 days. Feed intake and feed efficiency of each floor pen were estimated from the difference between the total quantity of feed distributed and the weight of the remaining feed at 7, 21 and 36 days.

### *Microbiological analyses*

For the study of the digestive flora, 15 animals from each treatment (3 per floor pen) were euthanized for faecal content sampling in order to follow the amount of total coliforms, *C. perfringens*, *Enterococcus* and Lactic acid bacteria at the rearing level. At the processing plant level, 15 samples of each treatment were taken from neck and thigh skins. On these samples, thermophilic coliforms, *S. aureus*, *Pseudomonas* and Lactic acid bacteria were enumerated the day of slaughter. After 7 days chilling at 4°C *Pseudomonas* and Lactic acid bacteria were enumerated on neck skins.

### *Statistical analyses*

The effects of the feed complement were tested using when possible the parametric test Anova followed by Tukey's test. When the conditions of normality and homogeneity of the variances are not completed, the non parametric test of Kruskal-Wallis was performed followed by Mann and Whitney's test in case of significant differences between the treatments

## RESULTS

### *Growth performances*

The administration of the lactic product did not show any significant effect ( $p=0.698$ ) between 0 and 7 days on the daily weight gain (DWG): the control and treated animals presented a DWG around 19 g/animal/day (table 1). Between 7 and 21 days, animals treated with 10<sup>5</sup> cfu/ml showed a significant higher DWG ( $p=0.090$ ) than the other animals. Despite the lack of statistical



signification, treated animals with  $10^6$  cfu/ml had a higher DWG than the control ones (table 1). During the last rearing period, between 21 and 36 days, all the animals receiving or not the product had a similar DWG: about 88 g/animal/day. Finally, the lactic product had a significant beneficial effect ( $p=0.049$ ) on the final body weight of the animals, but only at the dose of  $10^5$  cfu/ml: 2255g for T1, while 2235g for T2 and 2216g for T0.

**Table 1.** Animal daily weight gain (DWG) expressed in g/animal/day

Treatments	0–7 days	7–21 days	21–36 days
T0 (control)	19.5 ± 0.4	51.5 ± 1.6 (a)	87.9 ± 2.4
T1 ( $10^5$ cfu/ml)	19.1 ± 1.0	53.6 ± 1.1 (b)	88.6 ± 1.5
T2 ( $10^6$ cfu/ml)	19.2 ± 0.5	52.7 ± 1.0 (ab)	88.1 ± 2.5
	NS ( $p=0.698$ )	S ( $p=0.090$ )	NS ( $p=0.932$ )

Reported values (mean ± standard deviation; n=5) have been statistically analysed using the test of Kruskal-Wallis followed by Mann and Whitney's test. S: significant ( $p<0.05$ ); NS: non significant ( $p>0.05$ )

The treatment by the lactic product did not significantly affect either the feed gain ratio (FGR) (table 2), nor the feed consumption.

**Table 2.** Animals feed gain ratio (FGR)

Treatments	0–7 days	7–21 days	21–36 days
T0 (control)	1.15 ± 0.03	1.46 ± 0.03	1.79 ± 0.09
T1 ( $10^5$ cfu/ml)	1.17 ± 0.03	1.41 ± 0.04	1.76 ± 0.08
T2 ( $10^6$ cfu/ml)	1.15 ± 0.04	1.44 ± 0.01	1.78 ± 0.05
	NS ( $p=0.756$ )	NS ( $p=0.137$ )	NS ( $p=0.779$ )

Reported values (mean ± standard deviation; n=5) have been statistically analysed using the test of Kruskal-Wallis followed by Mann and Whitney's test. S: significant ( $p<0.05$ ); NS: non significant ( $p>0.05$ )

### Health status

In general, no major health problem did occur during the rearing period except a short term diarrhoea which appeared at the beginning of the rearing. A non typable *Escherichia coli* was found in the faeces in the same period. A visual analysis has been realised over 50 chicks per floor pen based on the distinction between spoiled animals from clean ones in order to estimate the number of animals which contracted the diarrhoea (table 3). The statistical analysis showed that the number of animals treated by  $10^5$  cfu/ml was significantly lower ( $p=0.031$ ) than the number of animals from the other treatments (table 3).

**Table 3.** Diarrhoea observed on chicks at the beginning of the rearing period

Treatments	Diarrhoea (% spoiled chicks)
T0 (control)	22 ± 13 (a)
T1 ( $10^5$ cfu/ml)	8 ± 8 (b)
T2 ( $10^6$ cfu/ml)	22 ± 8 (a)
	S ( $p=0.031$ )

Reported values (mean ± standard deviation; n=5) have been statistically analysed using the test of Kruskal-Wallis followed by Mann and Whitney's test. S: significant ( $p<0.05$ ) ; NS: non significant ( $p>0.05$ )

## MICROBIOLOGICAL ANALYSIS

### *At the rearing level*

The treatment by the lactic product did not affect the bacterial count of lactic acid bacteria, total coliforms, *Enterococcus* and *C. perfringens* as no significant differences have been observed between control and treated animals at any sampling period (table 4). However, there is a significant effect of the rearing period on the means of bacterial count. In general, for all the microorganisms studied, the lowest count was noted between 7 and 21 days of rearing (table 4).

**Table 4.** The means of bacterial count in animal faeces (log (10) cfu/g).

Microorganisms	Treatments	0–7 days	7–21 days	21–36 days
Lactic acid bacteria	T0 (control)	9.2 ± 0.6	7.7 ± 0.5	9.3 ± 0.3
	T1 (10 <sup>5</sup> cfu/ml)	9.2 ± 0.6	7.7 ± 0.6	9.2 ± 0.4
	T2 (10 <sup>6</sup> cfu/ml)	9.1 ± 0.4	7.9 ± 0.4	9.3 ± 0.6
		P=0.739		
Total coliforms*	T0 (control)	8.4 ± 0.4	7.5 ± 0.6	7.9 ± 0.6
	T1 (10 <sup>5</sup> cfu/ml)	8.0 ± 0.4	7.6 ± 0.6	7.8 ± 0.7
	T2 (10 <sup>6</sup> cfu/ml)	8.0 ± 0.5	7.6 ± 0.6	7.8 ± 0.6
		P=0.501		
<i>Enterococcus spp.</i>	T0 (control)	8.4 ± 0.4	7.5 ± 0.6	7.9 ± 0.6
	T1 (10 <sup>5</sup> cfu/ml)	8.0 ± 0.4	7.6 ± 0.6	7.8 ± 0.7
	T2 (10 <sup>6</sup> cfu/ml)	8.0 ± 0.5	7.6 ± 0.6	7.8 ± 0.6
		P=0.746		
<i>C. perfringens</i>	T0 (control)	4.2 ± 1.9	3.2 ± 1.2	5.0 ± 1.5
	T1 (10 <sup>5</sup> cfu/ml)	4.2 ± 0.5	2.5 ± 1.7	2.9 ± 2.1
	T2 (10 <sup>6</sup> cfu/ml)	4.0 ± 1.2	2.2 ± 1.0	4.1 ± 1.9
		P=0.057		

Reported values (mean ± standard deviation; n=15) have been statistically analysed using the test of Kruskal-Wallis or the \*Anova where the conditions of normality and homogeneity of variances were achieved. S: significant (p<0.05) ; NS: non significant (p>0.05)

### At the processing level

The animals from our experimental rearing have been slaughtered at the beginning of the process in order to avoid cross contamination with animals coming from other farms. The day of slaughtering (d36), the lactic product, had no effect on the counts of the microorganisms studied on neck skins taken from control and treated animals (table 5). The mean counts of lactic acid bacteria, *Pseudomonas* and thermophilic coliforms were 5, 3.7 and 4 log(10) respectively.

**Table 5.** The means of bacterial count on neck skins (log(10) cfu/g) the day of the slaughtering (d36).

Treatments	Lactic acid bacteria	<i>Pseudomonas spp.</i>	Thermophilic coliforms
T0 (control)	5,1 ± 0,4	3,8 ± 0,4	4,4 ± 0,8
T1 (10 <sup>5</sup> cfu/ml)	5,1 ± 0,3	3,8 ± 0,3	4,3 ± 0,6
T2 (10 <sup>6</sup> cfu/ml)	5,1 ± 0,3	3,7 ± 0,5	4,2 ± 0,5
	p=0.997	p=0.947	p=0.755

Reported values (mean ± standard deviation; n=15) have been statistically analysed using the test of Anova

The same analysis was done on thigh skins taken from control and treated animals (table 6). No significant effect of the lactic product has been recorded except for the count of thermophilic coliforms (table 6); however, from a biological point of view, the difference between the concentrations was considered not significant. The mean counts of lactic acid bacteria, *Pseudomonas*, *S. aureus* and thermophilic coliforms were around 4, 3, 2 and 3 log(10) respectively. The comparison of bacterial counts on neck and thigh skins showed a higher level on neck skins for the same microorganisms (tables 5 and 6).

**Table 6.** The means of bacterial count on thigh skins (log(10) cfu/g) the day of the slaughtering (d36)

Treatments	Lactic acid bacteria	<i>Pseudomonas spp.</i>	<i>S. aureus</i>	Thermophilic coliforms
T0 (control)	4.9 ± 0.3	3.3 ± 0.6	2.2 ± 0.3	3.9 ± 0.4
T1 (10 <sup>5</sup> cfu/ml)	4.6 ± 0.3	3.1 ± 0.5	2.3 ± 0.3	3.7 ± 0.3
T2 (10 <sup>6</sup> cfu/ml)	4.9 ± 0.3	3.3 ± 0.3	2.4 ± 0.4	4.4 ± 0.4
	P=0.180	P=0.197	P=0.266	P=0.000

The samples from thigh skins have been chilled at 4°C for 7 days (d43) in order to check the effect of the lactic product during the storage on the level of lactic acid bacteria and *Pseudomonas*. No significant effect of the lactic product was observed on the counts of lactic acid bacteria and *Pseudomonas* (table 8). At d43, the level of lactic acid bacteria decreased by about 0.5 log(10) compared to the level at d36 (tables 6 and 7). Nevertheless, the average count of *Pseudomonas* increased significantly from 3log(10) at d36 to 7log(10) at d43 (tables 6 and 7).

**Table 8.** The means of bacterial count on thigh skins (log (10) cfu/g) 7 days after chilling at 4°C (d43).

Treatments	Lactic acid bacteria	<i>Pseudomonas spp.</i>
T0 (control)	4.3 ± 0.4	6.9 ± 0.4
T1 (10 <sup>5</sup> cfu/ml)	4.4 ± 0.32	6.6 ± 0.5
T2 (10 <sup>6</sup> cfu/ml)	4.5 ± 0.2	7.2 ± 0.4
	P=0.468	P=0.146

## DISCUSSION

The growth performance of the chicken showed a significant effect, the lactic product added to the drink water at the dose of 10<sup>5</sup> ufc/ml (T1) compared to the control. However, no significant beneficial effect on the growth performance was observed at the dose of 10<sup>6</sup> ufc/ml (T2). The drinking water seemed yellowish at this higher dose, and it is likely that this high bacterial concentration altered the water quality, acting in turn on the intestinal flora of the birds and so on their growth performance.

If the lactic product at the dose of 10<sup>5</sup> ufc/ml improves the growth of the chickens, it seems that it improves also their health status. Indeed, even if a single opportunistic visual observation was made, it seems that the intake of this dose of the lactic product protected the birds against a short-time diarrhoea likely caused by a strain of *E. coli* at the beginning of the rearing period. The

visual observation indicated a lower proportion of animals affected by the diarrhoea and this protection could be related to the keeping of the balance of the intestinal flora.

The treatment had no effect on the studied digestive flora counts (lactic acid bacteria, total coliforms, *Enterococcus* and *C. perfringens*). In a same way, no significant effect between control and treated animals has been observed during the rearing period. The decrease in bacterial count at the period 7–21 days could be due to the changes in feed composition corresponding to feed transition periods.

At the processing level, the day of the slaughtering, the product had no effect on the level of the studied microorganisms (lactic acid bacteria, thermophilic coliforms, *Pseudomonas* and *S. aureus*) nor on neck skins neither on thigh skins as the comparison between control and treated animals showed no significant differences. During the storage period (7 days at 4°C) the product did not indirectly inhibit the growth of *Pseudomonas* on thigh skins as the level of these microorganisms was higher than the level the day of the slaughtering.

We conclude therefore that the lactic acid based product did not disturb the digestive flora tested during this trial and had no effect on the microorganisms recovered on neck and thigh skins at the processing levels. The benefit on *Salmonella* carriage reduction at the end of the rearing period in conventional herd condition will be performed.

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## INFLUENCE OF LEAD AND CADMIUM ON PRODUCTIVITY OF LAYING-HENS

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### SUMMARY

Investigation was conducted with 80 layers of ISA-Brown divided in 4 groups in 36 week age. The animal ration includes different amount of lead and cadmium: group 1-the amount of tow toxic elements is under the MRL. The amount of tow toxic elements in group 2, 3 and 4 is 10, 100, 1000 times bigger than MRL respectively.

Influence of lead and cadmium on productivity of laying-hens was studied. Decrease egg production, egg weight, eggshell weight, weight of yolk and albumen and consumption of fodder are established for high concentration of lead and cadmium. For low and median dose of lead and cadmium increase of egg production and decrease of consumption of fodder for 1 kg egg are noted.

**Keywords:** cadmium, lead, eggs, hens, productivity

### INTRODUCTION

Cadmium, an environmental pollutant is well to have a serious biological toxicity, and there have been many studies suggesting it to be the cause of "Itai-Itai disease" (Mochizuki et al 2002).

Lead is considered one of the major environmental pollutants; the effect of Pb on chicken, dove and wild animals is well-documented (Baykov et al 1996, Dong-Ha et al 2002, Medvedev et al 1999). Furthermore the ingestion of Pb by laying birds will result in an increase of Pb concentrations in eggs (Jeng et al 1997, Burger et al 2001). Information on the effect of Pb and Cd on laying hens is not available.

The objectives of this experiment were to investigate influence of lead and cadmium on productivity of laying-hens include laying capacity, egg mass, weight of egg, weight of white and yolk and weight of egg-shell.

### MATERIALS AND METHODS

Investigation was conducted with 80 hens of ISA-Brown's breed divided in 4 groups in 36 weeks age. The four groups are equalized by origin, sex and biomass. The birds ration includes different amount of lead and cadmium:

1. Group I-the amount of the tow toxic elements is under the MRL (Maximum Residues Limit).
2. Group II-the amount of the tow toxic elements is 10 times bigger than MRL.

3. Group III-the amount of the tow toxic elements is 100 times bigger than MRL.
4. Group IV-the amount of the tow toxic elements is 1000 times bigger than MRL.

Eggs were collected daily and laying capacity, weight of the egg, eggshell yolk and white were calculated every 15 days. Experiment continued 90 days.

For statistical analysis Origin® 7.0 SR0, V 7.0220 (B220) and Excel were used. The following variations of the analysis of variance (ANOVA) test were used for analysis of data. The criterion for significance was  $P < 0.05$

## RESULTS AND DISCUSSION

At analysis of results connected with laying capacity (table 1) were showed, that laying capacity decrease after first 15 days of experiment, which begin with 76.7% in control group and decrease to 74.3, 67.3 and 57% in II, III and IV respectively. After this time laying capacity decrease gradually and reach to 60.3% at 90 days. At higher dose of the Pb and Cd, lying capacity decrease in end the experiment to 15.1%. Similar results were obtained at dose of Cd 50 and 100 ppm (NRC 1980). In II group laying capacity increase comparatively with control group and reach at day 90 to 89.7%. Laying capacity in III group increase to middle of experiment (60 days) and decrease to end of the experiment.

**Table 1.** Laying capacity every 15 days (%)

Group	15	30	45	60	75	90	General medium
I	76,7±13,7	81,7±7.7	73 ±11.9	64,3 ±12.2	66,3 ±8.8	60,3±11.0	70,38±8.1
II	74,3±14,6	79,3±10.2	81,7±12.5	83,3 ±5.2*	86,7±5.2*	89,7±3.9*	82,5 ±5.4*
III	67,3 ±12,5	84,7±5.5	87,3±10.0*	87,7 ±5.3*	80,3±9.0*	79,7±5.6*	81,2 ±7.6*
IV	57±10,0*	56,3 ±9.7*	36,7±11.6*	47,7±10.2*	27,7±9.4*	15,1±10.0*	40,08±16.7*

\* significantly comparison with control group ( $P < 0.05$ )

Similar tendency at analysis of the results connected with egg mass is established (table2). Increase of the dose lead to decrease of egg mass only after the first 15 days. In the IV group decreasing of the egg mass vastly continued to end of the experiment, while in the II group egg mass increase gradually to end of the experiment. In the III group the egg mass increase to middle of the experiment (60 days) and decrease to end of the experiment. General egg mass in the end of experiment for all groups is 80.7, 92.9, 91.4 and 43.7 kg respectively.

**Table 2.** General egg mass every 15 days (kg)

group	15	30	45	60	75	90	General mass	General medium
I	14.61	15.51	13.97	12.49	12.58	11.50	80.66	13.44±1.50
II	13.79	14.80	15.36	15.80	16.29	16.84	92.88	15.48±1.09*
III	12.38	15.88	16.64	16.71	15.03	14.75	91.39	15.23±1.62
IV	10.47	10.24	6.69	8.70	5.05	2.56	43.71	7.29±3.27*

\* significantly comparison with control group ( $P < 0.05$ )

Important index is expense of the fodder for 1 kg egg mass. In the II and III expense of the fodder is 2.70 and 2.74 kg/kg egg mass, while in the control group is 3.09 kg. In the IV group expense of the fodder is 5.03 kg. Finley et al (1976) show that add of the lead to fodder of the duck with dose of 1, 5, and 25 ppm no lead to reliable difference in the consumption of the fodder after 12 month. Negative effect of the Pb and Cd on the productivity of the hens is established at analysis of the weight of egg, white, yolk and eggshell.

Medium egg weight decreased in to direction: at increase of the dose and continued time of the feeding (table 3). Medium egg weight in the control group was kept to end of the experiment (Jan et al 2004). General medium egg weight for I, II, III and IV is 63.68, 62.53, 62.49 and 60.66 g respectively. Similar tendency is noted at add Pb and Cd with tow dose low: 6.7 and 5.1 mg/l water respectively and high dose: 67 and 50 mg/l water respectively (Vodela et al 1997).

**Table 3.** Medium egg weight every 15 days (g)

group	15	30	45	60	75	90	General medium
I	63,51±3.77	63,3±4.96	63,8±3.31	64,7±3.00	63,21±4,6	63,55±3,44	63,68±3,85
II	61,84±5.91	62,2±4.53	62,7±3.11	63,2±4.36	62,64±4,24	62,6±4,02	62,53±4,36*
III	61,31±3.75	62,5±3.44	63,51±3.18	63,54±2.12	62,36±2,63	61,71±2,86	62,49±3,00*
IV	61,21±3.86	60,6±6.55	60,85±5.55	60,82±4,79	60,9±6,12	59,55±5,93	60,66±5,47*

\* significantly comparison with control group ( $P < 0.05$ )

Same tendency is noted at study of the white and yolk weight (table 4).

**Table 4.** Medium white and yolk weight every 15 days (g)

Group	15	30	45	60	75	90	General medium
I	57,1	56,3	57,3	57,8	56,3	56,6	56,9±0.60
II	55,9	55,8	56,5	56,6	56,1	56,2	56,2±0.32*
III	55,4	56,3	57,4	57	56,3	55,7	56,3±0.76
IV	55,3	55	54,5	54,6	55	54,1	54,8±0.43*

\* significantly comparison with control group ( $P < 0.05$ )

Influence of Pb and Cd is clear at study of the eggshell weight. Medium eggshell weight decrease at increase of the dose and at continued time of the feeding with exception of II and III groups after 60 days, where eggshell weight little decreased in comparison with previous periods (table 5).

**Table 5.** Medium eggshell weight every 15 days (g)

group	15	30	45	60	75	90	General medium
	6.37	6.99	6.52	6.89	6.91	6.93	6.77±0.25
	5.91	6.42	6.21	6.65	6.54	6.40	6.36±.026*
	5.92	6.23	6.11	6.54	6.10	6.01	6.15±0.23*
	5.95	5.58	6.33	6.19	5.90	5.50	5.90±0.33*

\* significantly comparison with control group ( $P < 0.05$ )

These results maybe connected with metabolism disturbance of calcium in the bird's organism. This be confirmed from Vodela et al (1997) which indicate that add Pb with dose 200 ppm lead to decrease laying capacity and calcium metabolism disturbance.

### CONCLUSION

Applying of the Pb and Cd in dose 10, 100 and 1000 time bigger than MRL lead to phasic effect on productivity of the hens and consumption of the fodder. Low dose of the Pb and Cd (II and III) lead to rise of laying capacity and received egg mass at low fodder expense. High dose of the Pb and Cd (IV) lead to decrease productivity and increase fodder expense.

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## **APOPTOSIS INDUCED BY COMMERCIAL FORMULATION OF CHLORPYRIFOS ON AVIAN LYMPHOCYTES**

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### **SUMMARY**

The present work was aimed at evaluating the toxic effects of the commercial formulation of chlorpyrifos (Radar 20% EC ) using avian lymphocyte model. Avian lymphocytes were treated with various dilutions of pesticide for 60 and 120 minutes. At the end of respective incubation periods, cells were harvested and analysed for lymphocyte proliferation and apoptosis assays. Results showed, the significant reduction of in both B and T cell counts suggesting the toxic effect of the pesticide. Apoptosis assays demonstrated the DNA fragmentation, phosphatidylserine translocation and plasma membrane blebbing in the pesticide treated cells. Based on the above findings it can be concluded that low dose of commercial formulation of chlorpyrifos is toxic that is mediated through the induction of apoptosis.

**Keywords:** chlorpyrifos, lymphocytes, apoptosis

### **INTRODUCTION**

Farming community routinely uses various commercial formulations of chlorpyrifos for the control of crop pests. During the time of pesticide application, farmers might get exposed to very low levels of that pesticide either due to unsystematic use or spillage etc. The toxic effects resulting from the exposure to chlorpyrifos depends upon the duration and dose to which animal, plant or humans get exposed. Although the very low dose of chlorpyrifos doesn't cause the apparent toxic signs to humans but appears that it might cause the invisible toxic effects and its residues could become potential environmental pollutants. The present investigation was aimed at examining the toxic effect of commercial formulation of chlorpyrifos using avian lymphocyte model.

### **MATERIALS AND METHODS**

Radar 20% EC (RPG Life Sciences Ltd, Mumbai, India); a commercial formulation of chlorpyrifos was procured from the local market and dissolved in ethanol to give 1% stock solution. In this study the pure form of chlorpyrifos was not used as this study sought to determine the toxic impact from exposures to the formulation of this agent that is routinely encountered by farmers and their livestock. The stock solution was serially diluted to ten folds using RPMI (Sigma, USA) maintenance medium. Avian (White leghorn) lymphocytes isolated from the blood were (final count adjusted to  $1 \times 10^7$  cells per ml) treated with 1:1000 dilution of chlorpyrifos and

incubated for 60 and 120 min at 37°C. At the end of each incubation period, cells were aliquoted into various groups for lymphocyte proliferation assay and apoptosis studies.

Lymphocyte proliferation assay was carried out as per the method of Rai-el-balhaa *et al.*, (1987). The reduction of the MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl-tetrazolium bromide) dye to formazan was used as an indicator of cell proliferation (Altman, 1976; Mosmann, 1983). The assay was carried out in the wells of 96-well plate. LPS (lipopolysaccharide) and Con-A (concanavalin-A) each were used at 5 µg/ml final concentration. The optical density (OD) was recorded at 570 nm using microscan ELISA reader (ECIL, India). The results were reported as mean change ( $\Delta$ ) in optical density (mean  $\Delta$  OD = mean OD of mitogen-stimulated wells – mean OD of unstimulated wells). DNA fragmentation assay was carried out as per the method described by Hermann *et al.*, (1994). The pellet was lysed using the lysis buffer (1% NP-40 in 20 mM EDTA, 50 mM Tris-HCl, pH 7.5), treated with sodium dodecyl sulphate (SDS), 10 M ammonium acetate, precipitated using ethanol and analysed upon agarose gel electrophoresis. Annexin-V binding assay was performed by the method of Chauhan and Tripathi (2002). Smears of cells quenched in 10% H<sub>2</sub>O<sub>2</sub> were stained with annexin-V-biotin and avidin-peroxidase conjugates. Slides were examined for cells displaying brown colour on their surface. Electron microscopy was done by the method of Malorni *et al.*, (1998) and ultra-structural changes were recorded.

## RESULTS AND DISCUSSION

Commercial formulations of chlorpyrifos are commonly used as organophosphate pesticides for the control of insects of paddy, cotton crops etc. Although they cause no apparent toxic signs following exposure to a very low dose, the adverse impact on the health of humans and animals cannot be ruled out. The present work was aimed at evaluating the toxic effects of very low dose (in this study 1:1000) of commercial formulation of chlorpyrifos using avian lymphocyte model. The mean  $\Delta$  in OD for con-A stimulated cells was 0.151±0.050 and 0.138±0.027 respectively at 60 and 120 min of pesticide exposure while for the control cells was 0.240±0.007 and 0.210±0.040 for the respective exposure times. The mean  $\Delta$  OD for LPS stimulated cells was 0.177±0.020 and 0.129±0.072 while for the control cells were 0.302±0.037 and 0.280±0.22 respectively after 60 and 120 min of exposure. The above result indicated the significant ( $p \leq 0.05$ ) reduction in mean  $\Delta$  in OD in chlorpyrifos treated cells when compared to the control group. The reduction in mean  $\Delta$  in OD was more at 120 min of exposure when compared to 60 min exposure. Among the treated group, the reduction in mean  $\Delta$  OD was more in con-A stimulated group in comparison to the LPS stimulated group. The reduction in mean  $\Delta$  in OD indicated the loss of viability of lymphocytes at that dilution. In addition, the data also suggested the T- cell specific toxicity of the chlorpyrifos. The reduction in mean  $\Delta$  in OD upon exposure to subsequent dilutions was not significant in comparison to the control group. The above result suggested that 1:1000 dilution of Radar 20% EC is toxic to lymphocytes. DNA fragmentation is the hall mark of the cells undergoing apoptosis. In the present study, to know chlorpyrifos caused toxicity on lymphocytes was due to apoptosis, DNA fragmentation assay was performed and the result revealed that chlorpyrifos induced the DNA fragmentation in avian lymphocytes. DNA fragmentation in lymphocytes appeared as DNA ladder on agarose gel. The intensity of DNA laddering was increased with the time as well as with the dose of exposure. To verify the apoptosis induced by chlorpyrifos, treated cells were assayed for another marker of apoptosis; phosphatidylserine exposure from the inner leaflet to the outer leaflet of plasma membrane (Vermes *et al.*, 1995).

Results demonstrated the translocation of phosphatidylserine to the outer leaflet as detected by the appearance of brown colour on the plasma membrane of the treated cells. This confirmed that the toxicity of chlorpyrifos on lymphocytes was due to apoptosis. In addition, the electron microscopy results also demonstrated the cells with condensed chromatin and plasma membrane blebbing.

Based on the appearance of DNA ladder, phosphatidylserine translocation to the outer leaflet, and the condensed chromatin in cells treated with chlorpyrifos, it can be concluded that commercial formulation chlorpyrifos (Radar 20% EC), even at very low dose cause toxicity in lymphocytes through the induction of apoptosis. The present study emphasizes the safe handling and systematic use of pesticides and also suggests that in-vitro techniques can be useful in toxicological evaluations of pesticides.

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## HEALTH AND WELFARE IN SWEDISH GAME BIRD REARING

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### SUMMARY

Rearing of game birds is a common practice in many European countries, and they constitute a large part of avian prey of hunters in these countries. In Sweden the size of the game bird rearing is partly unknown. The aim of the present study was to survey the extent of and the health and welfare of the game bird rearing in Sweden.

**Keywords** game birds, pheasant, mallard, grey partridge, animal health, animal welfare,

### INTRODUCTION

Rearing of game birds is a common practice in many European countries, and they constitute a large part of avian prey of hunters in these countries. It is e.g. reported that more than 1.5 million birds are released in Denmark annually and that these birds represent 1/3 of the hunters' prey. The main species for rearing are pheasants (*Phasianus* spp.), mallards (*Anas platyrhynchos*) and grey partridge (*Perdix perdix*), but there is also some rearing of quail (*Coturnix coturnix*), capercaillie (*Tetrao urogallus*) and black grouse (*Tetrao tetrix*). In Sweden the size of the game bird rearing is partly unknown. The lack of legislation in this area, concerns about the health and welfare of the game birds during rearing and outbreaks of avian influenza in countries around the world, make it important to collect more information about these activities. The aim of the present study was to survey the extent of and the health and welfare of the game bird rearing in Sweden.

### MATERIAL AND METHODS

A questionnaire was sent to all rearers who are registered by governmental authorities in Sweden, approximately 90. The questionnaire contained e.g. questions about number of adult birds and chickens at the facility, about housing, feeding and management routines of the birds, what kind of diseases and other problems there might be and if there were problems with predators at the setting out area.

### RESULTS

The majority of Swedish game bird rearers were found in the southern part of the country. The survey was answered by 72%. There were answers from 61 rearers of pheasant, mallard or grey partridge; 43 from pheasant rearers, 34 from mallard rearers and 27 from grey partridge rearers.

Of these, 30 kept one species, 19 kept two species and 12 kept three species. The main purpose of rearing as stated by the rearers is presented in Table 1. More than one answer was possible.

**Table 1.** Main purposes of game bird rearing

	Hunting	Dog training	Reinf.wild population
Pheasant	31	11	19
Mallard	28	3	13
Grey p.	11	15	7

### *Pheasant*

Of 37 facilities there was one (3%) from the middle of the 19<sup>th</sup> century, one (3%) from the 1960, 7 (19%) from the 1900–1940, 5 (14%) from the 1970–1980 and 23 (62%) from the 1990–2000.

Two rearers bought a total of 16,000 Swedish eggs. No one imported pheasant eggs. Twelve rearers bought 11,900 Swedish pheasant chicks. Breeding pheasants were kept at 25 facilities during breeding season and at 11 facilities during the rest of the year. The total number of breeding birds was 8179 during the breeding season and 2488 during the rest of the year. The median number of birds at each facility was 300 (min 23, max 700) during breeding season and 200 (min 23, max 600) during the rest of the year. The breeding pheasants were collected through purchase, catching wild birds and/or recruitment from own birds. A total number of 131,225 pheasant chicks were held at 33 facilities (median 4,000, min 50, max 12,000).

Median stocking density for breeding birds was 0,45 animals/m<sup>2</sup> (min 0,06, max 6,5, n=20). The corresponding number for chicks was 5 animals/m<sup>2</sup> (min 0,479, max 48, n=26). Twenty six rearers had environmental enrichment for their breeding birds and 35 for their chickens. For breeding birds, vegetation was mentioned in 24 cases and perches in 21 cases. The corresponding numbers for chicks were 29 and 27, respectively. Other things mentioned were straw bundles and sandpits. The breeding birds were fed commercial feed in all cases but one, where they got wheat, barley, oats and corn (n=24). The chicks were fed commercial feed, of which pheasant feed and turkey feed were mentioned. Twelve flocks (50%) of the breeding birds were given supplement to their feed, mostly egg- or seashell. Fourteen rearers gave supplement to the chicks, mostly chopped, boiled eggs. In addition, multivitamins and vegetation were mentioned for both adults and chickens.

Symptoms of gapeworm infection (*Syngamus trachea*) were reported in 21 facilities (n=36) with a prevalence of 5–70% (n=13). Diarrhoea was reported in nine facilities with a prevalence of 0,5–20% (n=6). Symptoms of respiratory disease and foot injury were each reported from one facility, prevalence 1% and 0,5% respectively (n= 34 and n=35 respectively). No one reported incidence of eye infection or increased mortality. Pecking occurred in 13 of 34 facilities with a prevalence of 0,01–10% (n=10). Of 35 answering, 30 used preventative measures against pecking. Anti-pecking device was used in 26 cases, some in combination with enriched environment. Other preventative measures that were mentioned were enriched environment, relocation of the pecker and some salt in the drinking water. Anthelmintics were used preventative and/or as treatment by 27 rearers. Fenbendazol and febantel were the medications used. Antibiotics (e.g. tetracyclines) were used preventative and/or as treatment by nine rearers. Coccidiostats (e.g. sulfaclozin) were used preventative and/or as treatment by 28 rearers. Production records were kept by 21 pheasant breeders and mortality was registered by 14, otherwise the records varied in content. Median

mortality was 1,45% (min 0, max 5, n=22,) among breeding birds and 5% (min 0,6, max 10, n=31) among chickens.

A total number of 94,279 pheasants were released from 38 facilities in 2005 (median 2,000, min 50, max 6,500).

### *Mallard*

Of 27 facilities two (7%) were started between 1900–1940, one (4%) in the 1950ies, 6 (22%) in the 1970–80ies and 18 (67%) between 1990–2000.

Nine rearers bought a total of 73,900 Swedish and 16,000 Danish eggs, and fifteen rearers bought 69,600 Swedish ducklings. Breeding mallards were kept at 7 facilities during breeding season and at 6 facilities during the rest of the year. The total number of breeding birds was 4000 during the breeding season and 3020 during the rest of the year. The median number of birds at each facility was 400 during both breeding season and the rest of the year (min 100, max 1000 and min 120, max 1000, respectively). The breeding mallards were collected through purchase, catching wild birds and/or recruitment from own birds. A total number of 151,400 ducklings were held at 24 facilities (median 4,000, min 200, max 30,000).

Median stocking density for breeding birds was 0,665 animals/m<sup>2</sup> (min 0,17, max 1,33, n=4). The corresponding number for ducklings was 5 animals/m<sup>2</sup> (min 1,33, max 55, n=19). Seven rearers had environmental enrichment for their breeding birds and 26 for their chickens. Vegetation was mentioned in 4 cases for breeding birds and in 14 cases for ducklings. Other things mentioned were egg nests, water and straw bundles for breeding birds and sand for ducklings. Twenty six rearers answered that the mallards had access to water for swimming. All breeding birds and ducklings were fed commercial feed (n=7 and n= 28, respectively). Four flocks of the breeding birds were given supplement, consisting of egg- or seashell and/or vegetation. Three rearers gave supplement, consisting of seashell, grass, gravel and/or multivitamin, to the ducklings.

Respiratory disease, eye infection, foot injuries and hysteria were each reported from one facility (n=28 in all four). The prevalence of respiratory disease was not stated. The other had prevalences of 0,5%, 2% and 0,5% respectively. No one reported incidence of diarrhoea or increased mortality in the facility. No rearer reported occurrence of pecking. Five used preventative measures against pecking in the way of enriched environment and feeding in several places. One breeder used anthelmintics with fenbendazol (n=29). Antibiotics and coccidiostats were not used by anyone (n=29 and n=28 respectively). Production records were kept by 19 mallard breeders and mortality was registered by 9, otherwise the records varied in content. Median mortality was 2% (min 0, max 5, n=6) among breeding birds and 1,5% (min 0,4, max 10, n=26) among ducklings.

A total number of 87,014 mallards were released from 31 facilities in 2005 (median 2,000, min 50, max 10,000).

### *Grey partridge*

Of 22 facilities two (9%) were started between 1900–1940, 5 (23%) in the 1970–1980ies and 15 (68%) between 1990–2000.

Two rearers bought a total of 500 Swedish eggs and 8000 Danish eggs (n=19). Nine rearers bought 3750 Swedish grey partridge chicks. Breeding partridges were kept at 10 facilities both during breeding season and during the rest of the year. The total number of breeding birds was 3834 during the breeding season and 3265 during the rest of the year. The median number of birds

at each facility was 220 (min 20, max 1600) during breeding season and 250 (min 20, max 1000) during the rest of the year. The breeding partridges were in all cases collected through own recruitment. A total number of 29,725 grey partridge chicks were held at 15 facilities (median 1,250, min 125, max 6,000).

Median stocking density for breeding birds was 2 animals/m<sup>2</sup> (min 0,8, max 9,4, n=8). The corresponding number for chicks was 4,1 animals/m<sup>2</sup> (min 0,95, max 31, n=16). Nine rearers had environmental enrichment for their breeding birds and 18 for their chickens. Breeding birds had vegetation or sand in five cases and a combination of vegetation and sand in two cases. Vegetation was mentioned in 15 cases and sand in 7 cases for chickens. Other things mentioned for chickens were perches, straw, wood shavings and gravel. Both breeding birds and chickens were fed commercial feed (n=9 and n= 20 respectively), and for chickens pheasant feed and turkey feed were mentioned by 6 and 7 respectively. Five of the flocks of breeding birds were given supplement to their feed, mostly egg- or seashell. Nine rearers gave supplement consisting of chopped, boiled eggs, eggshell, ant egg, vitamins and/or gravel to the chicks.

Symptoms of gapeworm were seen in 11 facilities (n=19) with a prevalence of 5–100% (n=7). Diarrhoea was seen in four facilities with a prevalence of 3–20% (n=19). Three facilities had episodes of hysteria with a prevalence of 0,5–4% (n=19). Symptoms of respiratory disease and foot injury were each reported from one facility, prevalence of 2% and 0,1% respectively (n= 20 on both). No one reported incidence of eye infection or increased mortality (n=20 on both). Pecking occurred in 8 of 20 facilities with a prevalence of 2–50% (n=5). Of 20 answering, 18 used preventative measures against pecking. Anti-pecking device was used in 15 cases, some in combination with enriched environment. Three used enriched environment as preventative measurement (of which two did not have pecking problems). Anthelmintics were used preventative and/or as treatment by 16 rearers. Fenbendazol and febantel were the medications used. Antibiotics (e.g. tetracyclines) were used preventative and/or as treatment by 5 rearers. Coccidiostats (e.g. sulfaclozin) were used preventative and/or as treatment by 10 rearers. Production records were kept by 14 breeders of grey partridge and mortality was registered by 9, otherwise the records varied in content. Median mortality was 1,25% (min 0, max 5, n=8) among breeding birds and 4,5% (min 0, max 15, n=18) among chickens.

A total number of 11,553 grey partridges were released from 19 facilities in 2005 (median 400, min 35, max 3,500).

## DISCUSSION AND CONCLUSION

Through clearer information about anonymity in the questionnaire, the number of answers could have been higher.

About 60–70% of the facilities were from the 1990–2000. One reason is probably new methods of dog training, which is evident for rearers of partridge, where the main purpose of rearing mainly was dog training.

A higher proportion of pheasant rearers kept breeding birds compared to rearers of mallards and grey partridges. On the other hand, most of the rearers that kept breeding mallards and partridges kept them all year round. The collection of birds was mostly through catching wild birds for pheasants and mallards, but only through own recruitment for partridge. Because of the increased risk of transmission of avian influenza from wild birds to domestic poultry, it is according to the Swedish National Board of Agriculture (SJV) since 2006 prohibited catching wild mallards. Number of purchased eggs and ducklings were much higher for mallards than the

corresponding numbers for pheasants and partridge. Although the number of buyers was not correspondingly larger which points at a larger purchase per rearer. This was also shown as there are fewer rearers of mallard but higher total number of birds, compared to pheasant. The number of rearers and the number of birds varied substantially between the three species. The rearers of mallards were nine *more* than the rearers of partridge and had 120,000 more birds, while they (mallards) were nine *less* than pheasant rearers, but had 20,000 more birds.

The stocking densities showed large variation, and this was probably because some stated the minimum stocking density (when the chickens had access to the whole area with breeding house, roofed outdoor area and the outdoor run) while others stated the maximum stocking density (when the chicks only had access to the breeding house). The stated maximum densities were higher than in the Swedish recommendation (Anonymous (2007a) for breeding birds of all three species and for ducklings. Cain (1984) has shown that feather pecking among pheasants was significantly reduced by increasing floor space per bird. Also environmental enrichment is important to prevent problems with pecking. What kind and what amount of environmental enrichment varied in and between species.

Almost all birds were given commercial feed. In the wild, pheasants and grey partridge chicks eat a diet mostly containing invertebrates. Commercial feeds are often low in fiber content but well balanced in nutrients, easy to use and the chickens grow fast (Liukkonen-Anttila, T, Putaala, A. and Hissa, R. 2002). However, studies have shown that galliform birds fed an artificial diet have shorter intestines than wild birds (Moss, R. 1972). It is suggested by Liukkonen-Anttila, T, Putaala, A. and Hissa, R. (2002) that gut morphology may affect the survival of the bird if reared for release into the wild, and it is therefore suggested to supplement the feed to grey partridge chicks with invertebrates during their first weeks of life. In our study, about 40% of pheasant chicks and 50% of grey partridge chicks were given supplement to the feed.

Mallards were in general healthy and sparsely medicated in this study. The most common diseases among pheasants and partridges were gapeworm and diarrhoea, which affected about 60% and about 25% respectively. Gapeworm is introduced through wild birds, and when the infection is established it is mainly spread through earth worm (Anonymous 2007b). The symptoms are respiratory problems and weakness, and it can be fatal through suffocation. Although it was common among the rearers in this study to use prophylactic or therapeutic medication against gapeworm, there is no registered drug for this in Sweden at the moment. Fenbendazol, used by rearers in this study, can be effective, but if there are many immature worms, and there is continued exposure, clinical relapse may occur, requiring a second treatment (Lister, S. 1993).

Around 50–60% of rearers kept records of their animals. Following the registration there is a requirement of keeping records of the animals.

In total, 192,846 game birds were released in 2005 by the game bird rearers in this study. In comparison, it was estimated that 1,5 million game birds were released in Denmark in 2004 (Clausen B. 2004) and that 40 million pheasants are released in England annually (Anonymous undated)

From the survey relevant facilities were selected for on-farm investigation. At these visits birds were clinically scored and management and environmental factors registered. These visits take place in May-June 2007.



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## A COMPARATIVE RISK CONSEQUENCES ASSESSMENT FOR AVIAN INFLUENZA OUTBREAKS OCCURRED IN ROMANIA

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### SUMMARY

Avian influenza is nowadays, an animal disease which has major implications for the public health and considerable direct and indirect social effects. It generates major losses in the poultry industry, plus significant collateral economic losses, for the time being.

The Service of Policies, Strategies, Programmes and Sanitary Veterinary Procedures within the National Sanitary Veterinary and Food Safety Authority together with the Projection, Development, Coordination and Research Service within the Institute for Diagnosis and Animal Health, performed a risk analysis concerning the wild bird risk for Romania, related to the avian influenza, in order to facilitate a early reaction of the sanitary veterinary and food safety services and of those for public health.

A first stage of avian influenza risk identification was issued related to the wild birds, especially to the migratory ones. The risk origin, the risk itinerary, the risk intermediate zones, the emission risk (concerning the introduction of the avian influenza into Romania), the exposure risk (of the wild and domestic birds to the avian influenza virus), and the transmission risk (in Romania and later for migration) were underlined here.

The identified risk was evaluated then after the place and dimensions of wild birds migrations and assessed upon the import critical unit values for the avian influenza.

This work aims at explaining last part of the risk identification — evaluation of risk consequences for the avian influenza outbreaks occurred in Romania in the three diseases waves identified during October 2005 — July 2006.

**Keywords:** avian influenza, risk analysis, risk identification, consequences of risk

### INTRODUCTION

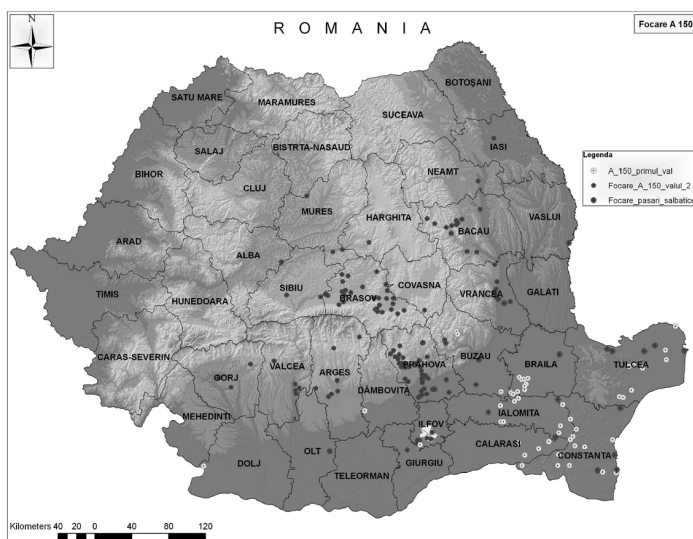
Romania was the first European and the most affected country by the avian influenza during the time 2005–2006.

A time assessment of the influenza outbreaks occurred in Romania points out that it developed in three waves, the first were correlated, while the third had apparently no connection with the first two.

The first wave of avian influenza lasted in October – December 2005 and consisted of 24 outbreaks in two counties in Eastern Romania, comprising the Danube Delta (Tulcea County) and the associated lakes to it and to Black Sea shore.

The second wave, with 30 outbreaks, was located in seven counties, around the first wave second counties. It had direct epidemiological correlation with the first wave outbreaks, but developed in January – March 2006.

The third wave had 132 outbreaks was spread over 19 counties, in May – June 2006 and, other than those affected by the two first bird flu waves, except two counties.



## MATERIAL AND METHOD

A comparative evaluation of the global and specific risk was elaborated for the three avian influenza waves in Romania. It was selected the Carvallo-Merkhofer estimation approach on the risk analysis structure, where the estimation of the risk consequences is a stage of the first domain of the risk analysis, with regard to the transmissible animal diseases.

A comparative estimation of avian influenza risk consequences, with respect to migratory birds was set up taking into account the direct sanitary veterinary effects of the disease, expressed mainly by the morbidity and mortality indices, as well as by the records of the disease indirect effects through veterinary expenses, intensive surveillance measures for prevention and control over the disease in the outbreak proper and avoiding dissemination of the diseases from the outbreaks into the territory.

The comparative estimation of avian influenza risk was performed globally, on the one hand, having in view both wild and domestic birds, and also specifically, with regard to certain birds types and species involved. Avian influenza risk consequences was focused to the impact of diseases collateral effects, i.e. upon the poultry sector, agriculture, poultry trade, hatching eggs trade, eggs for consumption and egg products or egg based products, upon tourism and last, but not least, upon the public health safety.

A qualitative estimation of avian risk consequences for Romania was then followed by the quantitative risk (quantified) estimation expressed in expenses or financial losses. The processed data are those obtained from the technical and financial records, related to the veterinary domain, further from the official addresses held by the employers' unions, in the poultry industry, official data from the local and central public administrations, involved municipalities and ministries.

## RESULTS

We identified at least six domains or major economic branches which were affected by the avian influenza: economical, trade, social, financial, cultural and public health.

Related to direct damages by morbidity and mortality, pursuant to data given by the international organisms in the field, during 1999–2001, when Italy was faced to the H<sub>7</sub>N<sub>1</sub> virus of the avian influenza, approximately 13 million poultry were killed or died. Around 17 million birds were killed, when the direct expenses thereto amounted to 62 million dollars, in the United States of America, after the incidence, in 1983, of the avian influenza caused in Pennsylvania by the H<sub>5</sub>N<sub>2</sub> virus, during the two years that lasted the disease.

Local authorities destroyed about one fourth of the domestic birds (about 30 million) during the epidemics with the H<sub>7</sub>N<sub>7</sub> virus that struck Netherlands, in 2003. In Belgium some 2.7 million were killed, and in Germany – 400.000 poultry. The Netherlands lost 150 million Euros during the episode of 2003.

In Romania, a number of 800,000 poultry were killed in the affected farms during the third wave, while in the householding yards 300,000 were killed, so reported by the Ministry of Agriculture, Forests and Rural Development.

Losses by death in wild birds, mainly in the Danube Delta were considerable. It was evaluated that approximately 150.000 birds of the sensitive species died, during the first avian influenza wave. Surprisingly some species that were not considered as being very receptive to avian influenza virus: wild pigeon, heron, bald coot, brown head duck, red neck goose were also affected by the virus. Greatest surprise, however, was the high mortality percentage registered in swans, confirmed by micro-outbreaks identified also in Europe. We now submit as being suggestive the losses registered by the Danube Delta Biosphere Reservation.

Summer swans and wild small hens dead in one month after the first case of avian influenza reported:

- 15–21.10.2005: 178 summer swans dead in the piscicultural arrangements Maliuc, Obretinul Mare and Popina, the lakes Obretinul Mic and Babina, canal Lopatna and casily flooded zone and top of the bank ridge Caraorman;
- 25.10.2005: 12 summer swans dead on the lake Obretinul Mic, 1 swan in the zone strict protégée Saraturi-Murighiol-Popina and 1 swan on the lake Ciulinet;
- 27.10.2005: 16 summer swans dead in the zone piscicultural arrangements Maliuc;
- 31.10.2005: 11 summer swans dead on the lake Taranova;
- 2.11.2005: 8 summer swans dead, from wich 5 juvenile, on the lake Obretinul Mic;
- 4.11.2005: 4 summer swans dead in the Gulph Musura;
- 7.11.2005: 12 summer swans dead, from which 6 juvenile, in the lake Obretinul Mic;
- 8.11.2005: 20 summer swans dead in the Gulph Musura;
- 9.11.2005: 3 summer swans dead on the lake Fortuna;
- 10.11.2005: 6 summer swans dead and one small hens on the lake Obretinul Mic;
- 11.11.2005: 23 summer swans dead, from which 17 juvenile, on the lake Taranova;
- 13.11.2005: 2 summer swans dead on the lake Fortuna and other 2 on the lake Balcanesti;
- 15.11.2005: 6 summer swans dead in the Gulph Musura;
- 16.11.2005: 7 summer swans dead on the lake Obretinul Mic, other 2 in the Gulph Musura and 9 in the canal of infiltration between maritime mile 14 – maritime mile 15, on the canal Sulina
- 23.11.2005: 6 summer swans dead (from which 5 juvenile) on the lake Capcicova;
- 25.11.2005: 16 summer swans dead on the lakes Obretinul Mic and Capcicova.

Trading consequences were strikingly resented in the field of business, domestic markets, sales of poultry and poultry products, destruction of consumers' confidence, caused by the drastic reduction of poultry, egg and poultry products consumption due to the restriction imposed on both inland and export market. It was estimated that the effect of the bird flu led to a decrease of 85% on the poultry sales that caused a 60% loss for the poultry farmers and operators on poultry products.

The total losses due to closing animal markets and fairs (determined by the sanitary veterinary and food safety authorities and by the local administration authorities) and to the restrictions on the movement of certain species of animals, were estimated at 40–50 million euros.

Losses generated by the export bans imposed to Romania by the European Commission and the Member States, and also by third countries, with which Romania had economic relationship in the poultry domain, were assessed as being 13 million euros, only for the first wave, during October – December 2005, in the two counties. It is estimated that the total losses for the three waves were 62 million euros in this domain only.

Social problems were induced by applying the stamping-out procedure for the first time on such a large scale in Romania, in the affected areas, by an emotional effect and, especially because the killed poultry, was virtually the sole (and main) meat source for the population in the harmed zones. More that, the quarantine rules imposed on the outbreaks spots and around them, sometimes even for humans, the trouble for agricultural and other activities in the countryside, led to a hostile behaviour against local and central authorities, towards vets and law enforcement organs that applied the veterinary measures and restrictions. Romania's Government spent 5 million euros for helping population pass over this profound crisis, to supply them food and health protection means.

The losses in the poultry sector were tremendous. Data furnished by the poultry sector employers outline that the damages due to non population in accordance with the technologic flow with 3.07 million chicken amount to 288.3 billion old Romanian lei, i.e. 8,479,411.76 euros. Losses due to the delays put on chicken cutting caused, by the imposed restrictions, amount to 12.3 billion old Romanian lei, i.e. 361,764.70 euros. Further, losses caused by export ban are 170.2 billion old Romanian lei – 5,005,882.35 euros.

Moreover, losses due to poultry meat price diminishing at slaughterhouse door – 504.1 billion old Romanian lei, which equal 4,826,470.60 euros. Besides we can mention, losses due to non using the hatching eggs, that mean 9.6 billion old Romanian lei, equivalent to 282,352.90 euros, additional costs by stocking the banned meat – 226.8 billion old Romanian lei, i.e. 6,670,588.20 euros, additional costs with disinfection – 24.1 billion old Romanian lei, 708,823.50 euros, where the overall losses were in the sector 1,235.4 billion old Romanian lei, i.e. 363,352,940.10 euros.

Increase of the poultry meat consumption in 2005 by 152% for the first nine months, until outbreaks of the avian influenza, resulted in an increase of 512% of poultry meat import, especially for the last two months of the year.

Financial implications consisted mainly in expenses involving application of sanitary-veterinary measures, financial compensations granted to the affected people and losses in the banking sector.

In order to apply veterinary restriction measures, the Government of Romania spent 23 million euros in the first disease wave only. The compensations for having killed the poultry amounted to 4 million euros. Disinfection operations meant 40 million euros expenses dedicated to the Ministry of Transports.

Bank sector losses were assessed to about 100 million euros due to the inability of the poultry sector to return credits entered into, necessary for its running.

Economic consequences of the bird flu during the time October 2005 – end of July 2006, were estimated at 120 million euros. Hunting sector was affected by approximately one million euros due to the interdiction on it. Tourism, especially the Danube Delta one, was affected with 10 million euros, while the number of tourists decreased by 90%, compared to contracts concluded.

Cultural and traditional activities were prohibited in the affected areas and surroundings. The efforts to protect public health related to the avian influenza have cost 17 million euros plus 2 million euros to ensure 5 million antinflu vaccine doses.

## CONCLUSIONS

1. The evolution of the avian influenza in Romania resulted in huge losses and extra expenses.
2. A number of at least six economic sectors directly and severely affected by the disease were identified.
3. The avian influenza generated a deep crisis in the poultry sector.
4. The considerable costs induced by the disease evolution mean a support to strengthen the surveillance capacity and rapid and early reaction of the sanitary veterinary authorities.
5. The necessity to implement a national wide management of crisis situations, with introduction of adequate structures in the sanitary veterinary was underlined.

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## POSTER PRESENTATIONS

### BIOACCUMULATION AND DISTRIBUTION OF LEAD AND CADMIUM IN HEN'S ORGANISM

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#### ABSTRACT

Investigation was conducted with 80 hens of ISA Brown's breed divided in 4 groups in 36 weeks age. The animal ration includes different amount of lead and cadmium: group 1-the amount of tow toxic elements is under the MRL. The amount of tow toxic elements in group 2, 3 and 4 is 10, 100, 1000 times bigger than MRL respectively (Pb=0.2, Cd=0.1 mg/kg).

Distribution of elements was studied with criteria: Klark of distribution (Kd) which is the ratio between the concentration of chemical element in mg/kg product and middle Klark (in wet weight). Criteria "Klark of distribution" allow to assessment of the bioaccumulation and distribution of the lead and cadmium in different organ and tissue.

**Keywords:** bioaccumulation, distribution, cadmium, lead, hens

#### INTRODUCTION

Heavy metals derive form industrial sources, natural erosion and geochemical cycles. Pollutants such as lead and cadmium enter the food chain through air and water. In estuaries, industrial and other anthropogenic sources often provide the primary source of heavy metals (Burger et al 2001, Dong-Ha et al 2002). There have been numerous studies of bioaccumulation in variety of organisms including mammals and birds. These studies usually examine levels of lead and cadmium in variety organs and tissues (Wayland et al 2001, Medvedev et al 1999, Erdogan et al 2005, Gotal et al 2992).

The amount of lead and cadmium which distributes in the organs and tissues of the animals depends on the interval of exposure, the quantity ingested; the production and reproduction phase of the animals, as well as their age and breed (Baykov et al 1996).

The dates in literatures have not criteria for assessment distribution of lead and cadmium in the organs and tissues of the animals.

The objective of our study was to determine cadmium and lead concentration in selected organs and tissues of the hens, and to estimate of the distribution dynamics of the lead and cadmium in the organism through criteria (suggested by us) that is Klark of distribution (Kd).

## MATERIALS AND METHODS

Investigation was conducted with 80 hens of ISA-Brown's breed divided in 4 groups in 36 weeks age. The four groups are equalized by origin, sex and biomass. The birds ration includes different amount of lead and cadmium:

1. Group I-the amount of the tow toxic elements is under the MRL (Maximum Residues Limit).
2. Group II-the amount of the tow toxic elements is 10 times bigger than MRL.
3. Group III-the amount of the tow toxic elements is 100 times bigger than MRL.
4. Group IV-the amount of the tow toxic elements is 1000 times bigger than MRL

Before analysis the samples were kept at  $-18^{\circ}\text{C}$ . in the laboratory the samples were weighted (2 g) and ashed with diluted nitric acid p.a. ( $\text{HNO}_3:\text{H}_2\text{O} = 2:1$ ) at  $130^{\circ}\text{C}$  for 2 h. undissolved particles were filtered off and the solution diluted to 25 ml. the digested samples were analyzed for the presence of cadmium and lead by using an atomic absorption spectrophotometer (AAS). The sensitivity for cadmium and lead was 0.0001 and 0.0005 mg/l respectively.

The dynamics of distribution was studied using criteria: Klark of distribution (Kd), which is the ratio between the concentration of chemical element in mg/kg product and medium concentration of the same chemical element in the organism.

For statistical analysis Origin® 7.0 SR0, V 7.0220 (B220) and Excel were used. The following variations of the analysis of variance (ANOVA) test were used for analysis of data. The criterion for significance was  $P < 0.05$

## RESULTS AND DISCUSSION

Data is presented in Table 1 and 2 for the content of the lead and cadmium in the organs and tissues of the hens. Metal concentrations were reported as mg/kg wet weight. Mean lead concentration were highest in bone in experimental group with dose 100 (1.097 mg/kg) and 1000 (16.415 mg/kg) fold bigger MRL ( $P < 0.05$ ), but the lower concentration is in the muscles and the heart. Feathers in comparison include high concentration of the lead. The concentration of Cd in the all organs and tissues differed significantly ( $P < 0.05$ ). The higher content of Cd in the kidney was established (1.567, 3.787, 13.983 and 102.660 mg/kg respectively in the four groups) (table 2) (Doganoc et al 1996, Grue et al 1984, Jeng et al 1997).

The Cd concentration in the lever of hens increase significantly ( $P < 0.05$ ) from 0.825 for control group to 47.750 mg/kg wet weight for IV-group (table 2). The higher content of the Cd in the feathers is 2.107 mg/kg in the IV group. The concentration of Cd in the muscles exceed MRL (0.05) in the experimental groups, which are with 100 and 1000 fold bigger MRL while in the II group is under MRL. The lower concentration of the Cd were in the muscles, where is 0.029, 0.038, 0.068 and 0.826 mg/kg wet weight respectively for the four groups. Same results were established in the rabbits, roe deer and mousse (Bilal et al 2003, Fenley et al 1979, Korenekova et al 2002, Lisunova et al 2003).

The analysis of the data in the literatures show only establishment of the contents of the lead and cadmium in the vary animal's organs and tissues (Erdogan et al 2005, Gotal et al 1992, Wayland et al 2001, Medvedev et al 1999), but no information for the real distribution dynamics of lead and cadmium in the animal's organism, for that reason we suggest application new criteria Klark of distribution, which is show distribution dynamics of the lead and cadmium, which enter



in the organism through fodder and water in the vary organs and tissues of animals or birds, which are object of the our study.

Data for Klark of distribution of lead and cadmium are presented in table 3 and 4 respectively. Medium Klark of the lead in the four groups increase with increase it level in the fodder. The analysis of results in the table 3 show tows directions, decrease and increase of the values of Kd with increase of the dose. Kd in the lever, kidney increases gradually. The Kd of lead in lever of hens for control group is 0.291 and increase to 0.333, 0.316 and 0.385 respectively for experimental groups. Data for kidney is 0.236, 0.727, 0.662 and 0.418 respectively for I, II, III and IV.

The Klark of distribution of lead in the lungs, heart, gizzard muscles, bones and the feathers decrease according with increase of the dose in fodder proportionally (table 3).

Medium Klark of the cadmium in the organs and tissues increase according with increase of the dose in the fodder. Data for distribution of the cadmium is presented in table 4. The values of Kd of cadmium in lever begin with 8.777 for control group and increase to 11.815 in II group, afterwards decrease to 10.457 and 10.789 in III and IV group comparison with II group. The Kd of Cd in the muscles, feathers, lungs and the bones decrease proportionally for all groups (table 4). In remaining organs and tissues (heart and skin) the values of the Kd are increased with several characteristics for every organ and tissue.

The Kd of Cd in the heart of hens from control group is 0.223 and increase to 0.175, 0.218 and 0.219 respectively for II, III and IV experimental groups. For the skin, Kd in the control group is 0.255 and increase to 0.260 in II group, but this value decrease to 0.246 in III group and increase to 0.350 in IV group.

## CONCLUSIONS

1. The concentration of lead and cadmium increase in the organs and tissues of the hens according with increase of the dose in fodder.
2. Criteria "Klark of distribution" allow to assessment of the bioaccumulation and distribution of the lead and cadmium in different organ and tissue.
3. The differences of steppe of the distribution of lead and cadmium give new explanations for bioaccumulation of tow toxic elements, respectively for importance in its accumulation in the studded organs and tissues, which connected with the mechanism of effect on kidney and bones (Itai Itai disease) and unfavorably effect on the function of heart.

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**Table 1.** Lead content in organs and tissues of hens (mg/kg wet weight)

	I	II	III	IV
<b>Liver</b>	0.016 ± 0.003	0.033± 0.004*	0.073± 0.017*	0.721 ± 0.72*
<b>Kidney</b>	0.013± 0.003	0.072± 0.048	0.153± 0.081*	0.784± 0.432*
<b>Lungs</b>	0.027 ± 0,019	0.065±0,019	0.110 ± 0,026	0.606 ± 0,244
<b>Heart</b>	0.018 ± 0,007	0.026± 0,006	0.053± 0,021	0.062 ± 0,027*
<b>Gizzard</b>	0.026± 0.005	0.031± 0.006	0.053± 0.032	0.172± 0.054*
<b>Muscle</b>	0.030±0,008	0.037± 0,010	0.040±0,011	0.055 ± 0,003*
<b>Skin</b>	0.02± 0.003	0.153± 0.037	0.225± 0.144	0.342± 0.156*
<b>Bones</b>	0.124 ± 0,019	0.178± 0.022*	0.980 ± 0.048*	14.656 ± 4.708*
<b>Feather</b>	0.183±0.044	0.433± 0.276	0.718± 0.261*	0.886± 0.091*
<b>Fodder</b>	0.45	1.13	9.95	95.48

\* significantly comparison with control group (P < 0.05)

**Table 2.** Cadmium content in organs and tissues of hens (mg/kg wet weight)

	<b>I</b>	<b>II</b>	<b>III</b>	<b>IV</b>
<b>Liver</b>	0.825 ± 0.179	2.363± 0.537*	4.120± 1.200*	47.750 ± 9.993*
<b>Kidney</b>	1.567± 0.059	3.787± 0.200	13.983± 2.948*	102.660± 18.428*
<b>Lungs</b>	0.153 ± 0,039	0.217±0,030	0.237 ± 0,014*	1.153 ± 0,110*
<b>Heart</b>	0.021 ± 0,007	0.035± 0,015	0.086± 0,022*	0.969 ± 0,043*
<b>Gizzard</b>	0.593± 0.073	1.287± 0.227*	1.567± 0.862*	5.807± 1.519*
<b>Muscle</b>	0.029±0,014	0.038± 0,012	0.068±0,040	0.826 ± 0,084*
<b>Skin</b>	0.024± 0.013	0.052± 0.003*	0.097± 0.046*	1.547± 0.105*
<b>Bones</b>	0.034 ± 0,007	0.091± 0.071	0.110 ± 0.037*	0.915 ± 0.298*
<b>Feather</b>	0.253±0.015	0.424± 0.098	0.866± 0.130*	2.107± 0.408*
<b>Fodder</b>	0.38	1.01	6.21	68.87

\* significantly comparison with control group (P < 0.05)

**Table 3.** Klark of distribution of lead in hen's organs and tissues (Kd)

	<b>I</b>	<b>II</b>	<b>III</b>	<b>IV</b>
<b>Liver</b>	0.291	0.333	0.316	0.385
<b>Kidney</b>	0.236	0.727	0.662	0.418
<b>Lungs</b>	0.491	0.657	0.467	0.323
<b>Heart</b>	0.327	0.263	0.229	0.033
<b>Gizzard</b>	0.473	0.313	0.229	0.092
<b>Muscle</b>	0.545	0.374	0.173	0.029
<b>Skin</b>	0364	1.545	0.974	0.182
<b>Bones</b>	2.255	1.798	4.242	7.817
<b>Feathers</b>	3.327	4.374	3.108	0.473

**Table 4.** Klark of distribution of cadmium in hen's organs and tissues (Kd)

	<b>I</b>	<b>II</b>	<b>III</b>	<b>IV</b>
<b>Liver</b>	8.777	11.815	10.457	10.789
<b>Kidney</b>	16.670	18.935	35.490	23.195
<b>Lungs</b>	1.628	1.085	0.602	0.261
<b>Heart</b>	0.223	0.175	0.218	0.219
<b>Gizzard</b>	6.309	6.435	3.977	1.312
<b>Muscle</b>	0.309	0.190	0.173	0.187
<b>Skin</b>	0.255	0.260	0.246	0.350
<b>Bones</b>	0.362	0.455	0.279	0.209
<b>Feathers</b>	2.691	2.120	2.198	0.476

## **EFFECT OF DELAYED CHICK PLACEMENT AND VARIED REARING TEMPERATURES ON BROILER CHICKEN PRODUCTIVITY**

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### **SUMMARY**

The aim of the study was to determine the effect of a 24-hour delay in chick placement and varied rearing temperatures to 21 days of rearing on the productivity of broiler chickens. Both reduced and increased air temperature during the first period of rearing reduced the rate of growth, with clearer differences observed in the group of birds reared at lower temperature. However, growth compensation occurred at a later period and birds from all the groups achieved similar final body weights. Different thermal conditions of rearing did not affect feed conversion, carcass quality or bird survival. The results obtained suggest that chickens exposed to a 24-hour feed and water withdrawal can cope with both reduced and elevated temperatures of rearing, with no effect on the production results obtained.

**Keywords:** broiler chickens, housing time, thermal conditions, productivity

### **INTRODUCTION**

The advancement of genetic progress in birds is paralleled by an increase in their productive value and reduced resistance to stress. Considering the welfare regulations that limit the quantity and quality of stressors, increasing importance in poultry practice is attached to the handling of chicks during the first hours after hatching. In theory, the time from hatching to the placement of chicks in a poultry house is very important. This concerns mainly hatchery and broiler house conditions and the time and conditions of chick transportation from the hatchery to the farm. Chicks hatched in the first stage are kept in difficult thermal and humidity conditions of the hatchery for around 24 or even 48 hours (Decuypere et al., 2001). Ton et al. (2003) reported that even chicks originating from one group do not hatch at the same time, which results in different chick quality. This time is extended by sorting (i.e. selection of birds), vaccination, preparation for transport and the transport itself (Pietras et al., 1990). These procedures may expose birds to stress reactions related mainly to social and thermal conditions (Herbut et al., 1993; Pietras et al., 1990) and to body dehydration, because during this period chicks have no access to water or feed. In the initial period of rearing, appropriate climatic conditions are particularly important because the thermoregulatory system of chicks is undeveloped and they are unable to compensate large heat losses in exceedingly low ambient temperatures or to lose excess heat in overheated facilities.

The aim of the study was to determine the effect of a 24-hour delay in chick placement and varied rearing temperatures to 21 days of rearing on the productivity of broiler chickens.

## MATERIAL AND METHODS

After weighing and tagging, a total of 270 day-old Ross 308 broiler chicks were assigned to one of three groups (control group I and experimental groups II and III), with 90 birds group. Chicks from all the experimental groups were simultaneously removed from the hatchery and transported for approximately 2 hours to an experimental broiler house of the National Research Institute of Animal Production. Prior to placement, birds were subjected for 24 hours to cold stress (ambient temperature 22°C), high stocking density and withdrawal of water and feed.

In the next stage of the experiment, chickens from group I were kept in standard temperature. To 21 days of rearing, air temperature was reduced by 5°C in group II and increased by 5°C in relation to the recommended temperature on the same days in group III.

Chickens were reared in 6-tier batteries of heated cages to 21 days of age and in 4-tier batteries of unheated cages to 42 days of age. Birds were kept in tiers of 30 chickens (2 subgroups) at a stocking density of 15 birds/m<sup>2</sup> and fed *ad libitum* with concentrate-based standard diets. During rearing, light intensity of 5–15 lx (depending on chicken age) and standard lighting program were used. Throughout the study, basic parameters of climate (temperature, air humidity, dry cooling of air and air movement) were measured. Individual body weight, feed intake and mortality were recorded every week. At 42 days of rearing, 20 chickens (pullets and cockerels) of average body weight were selected from each group. After slaughter and cooling, they were subjected to a simplified slaughter analysis.

The results were analysed statistically by way of analysis of variance and significance of differences was estimated using Duncan's test.

## RESULTS AND DISCUSSION

On the first day of rearing, chicks from the experimental groups were characterized by significantly greater body weight compared to the control birds [Tab. 1].

According to many authors, elevated and reduced air temperatures during rearing have a negative effect on weight gains and final body weights of broiler chickens (McGovern et al., 2000; Sokołowicz et al., 2000; Temim et al., 2000; Sosnowka-Czajka et al., 2001; Mashaly et al., 2004). In our study, elevated and reduced rearing temperature coupled with a 24-hour delay in placement also reduced the growth rate of broiler chickens. From 7 to 28 days of rearing, chicks from group II showed the lowest body weight ( $p \leq 0.01$ ). Chickens from group III were characterized by lower body weight compared to the control group only on day 7 of the study ( $p \leq 0.05$ ). However, in the subsequent period of the experiment, growth compensation occurred and to 35 days no differences in the body weight of chickens were found between the groups studied. This is consistent with the findings of Yahav and Hurwitz (1996) and Yahav and Plavnik (1999), who reported that reduced weight gains as a result of elevated air temperature are compensated in the later period of rearing and even exceed those of the control groups as a result of growth compensation. The process of growth compensation was also confirmed by Sokołowicz and Herbut (2001). Baarendse et al. (2006) reported that rearing chicks during the first five days of life at 28°C has a long-term negative effect on their further growth and development.

The use of a 24-hour delay in chick placement in the present study did not affect the final body weight of the birds because at 42 days of rearing they achieved an average of 2.4 kg body weight with feed intake of 1.9 kg/kg body weight, which is comparable with the results obtained by other authors (Herbut and Sokołowicz, 2004; Herbut et al., 2005).

In the present experiment, no effect of cold and heat stress on feed conversion by broiler chickens was found [Tab. 2], as confirmed by May and Lott (2000) and Sosnówka-Czajka and Herbut (2001). However, the scientific literature contains many papers that point to poorer feed conversion by broiler chickens exposed to thermal (cold or heat) stress (McGovern et al., 2000; Temim et al., 2000).

Thermal stresses applied in the present study did not affect bird mortality [Tab. 3]. Very low mortality in the first period of rearing could result from the good utilization of yolk sac stores and maternal antibodies, which had an effect on the health of broiler chickens. It is worth noting the favorable effect of conditioning chicks with high temperature in the first days of life on bird survival, as confirmed by the studies of Sokołowicz and Herbut (2001) and Sosnówka-Czajka and Herbut (2001).

According to many authors, thermal stress has a negative effect on the quality of broiler carcasses (Yalcin et al., 1999; Temim et al., 2000), but this was not confirmed by our study, where the experimental factors did not have a marked effect on carcass quality [Tab. 4]. Also Połtowicz and Sosnówka-Czajka (2005) found no effect of thermal stress on the quality of broiler carcasses and meat. However, improved carcass quality under cold or heat stress was reported by McGovern et al. (2000) and Sosnówka-Czajka and Herbut (2001, 2003).

## CONCLUSIONS

Reduced and elevated air temperature during the first period of rearing reduced the rate of growth, with clearer differences observed in the group of birds reared at lower temperature. Growth compensation occurred at a later period and birds from all the groups achieved similar final body weights. Different thermal conditions of rearing did not affect feed conversion, carcass quality or bird survival.

The results obtained suggest that chickens exposed to a 24-hour feed and water withdrawal can cope with both reduced and elevated temperatures of rearing, with no effect on the production results obtained.

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**Table 1.** Body weight of broiler chickens (g)

Days of rearing	Group		
	I	II	III
1	40.25 ± 0.34 a	41.80 ± 0.34 b	41.87 ± 0.33 b
7	153.94 ± 1.41 Cc	133.58 ± 1.42 Aa	144.27 ± 1.38 Bb
14	410.81 ± 3.77 Bb	367.04 ± 3.80 Aa	401.60 ± 3.70 Bb
21	764.13 ± 7.83 Bb	711.97 ± 7.88 Aa	748.27 ± 7.67 b
28	1353.89 ± 16.15 Bb	1263.24 ± 16.26 Aa	1306.13 ± 15.82
35	1896.53 ± 24.01	1840.14 ± 24.18	1834.00 ± 23.53
42	2390.28 ± 34.43	2355.63 ± 34.67	2325.33 ± 33.73

**A, B** – values in rows marked with different letters differ highly significantly ( $p \leq 0.01$ )

**a, b** – values in rows marked with different letters differ significantly ( $p \leq 0.05$ )

**Table 2.** Feed conversion (g/kg weight gain)

Days of rearing	Group		
	I	II	III
1-7	864 ± 22.8	910 ± 22.8	916 ± 22.8
8-14	1498 ± 17.6	1536 ± 17.6	1490 ± 17.6
15-21	1766 ± 36.6	1616 ± 36.6	1684 ± 36.6
<b>1-21</b>	<b>1490 ± 16.1</b>	<b>1462 ± 16.1</b>	<b>1464 ± 16.1</b>
22-28	1670 ± 70.4	1796 ± 70.4	1804 ± 70.4
29-35	2032 ± 71.7	1972 ± 71.7	2090 ± 71.7
36-42	2522 ± 78.7	2420 ± 78.7	2442 ± 78.7
<b>22-42</b>	<b>2036 ± 25.8</b>	<b>2050 ± 25.8</b>	<b>2096 ± 25.8</b>
<b>1-42</b>	<b>1862 ± 18.6</b>	<b>1868 ± 18.6</b>	<b>1896 ± 18.6</b>

**Table 3.** Chicken mortality (%)

Days of rearing	Group		
	I	II	III
1-7	0	0	0
8-14	1.33	0	0
15-21	0	0	0
<b>1-21</b>	<b>1.33</b>	<b>0</b>	<b>0</b>
22-28	0	0	0
29-35	1.33	4.00	0
36-42	4.00	2.67	5.33
<b>22-42</b>	<b>5.33</b>	<b>6.67</b>	<b>5.33</b>
<b>1-42</b>	<b>6.67</b>	<b>6.67</b>	<b>5.33</b>

**Table 4.** Results of slaughter analysis in 42-day-old broiler chickens (%)

Item	Group		
	I	II	III
<b>Dressing percentage:</b>			
▪ with giblets	75.22 ± 0.55	75.51 ± 0.55	75.93 ± 0.55
▪ without giblets	71.39 ± 0.57	71.63 ± 0.57	72.23 ± 0.57
<b>Muscles:</b>			
▪ breast	24.56 ± 0.41	24.29 ± 0.41	25.17 ± 0.41
▪ leg	20.52 ± 0.34	20.57 ± 0.34	20.67 ± 0.34
<b>Abdominal fat</b>	2.49 ± 0.16	2.45 ± 0.16	2.31 ± 0.16
<b>Giblets</b>	5.09 ± 0.13	5.14 ± 0.13	4.89 ± 0.13
<b>Liver</b>	3.26 ± 0.10	3.32 ± 0.10	3.07 ± 0.10
<b>Gizzard</b>	1.18 ± 0.05	1.09 ± 0.05	1.09 ± 0.05
<b>Heart</b>	0.65 ± 0.03	0.74 ± 0.03	0.74 ± 0.03



## INFLUENCE OF THE LIGHT REGIMEN ON BROILER GROWTH

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### SUMMARY

The experiment monitored the influence of the light regimen on broilers reared on the floor on permanent litter. The experimental period was 49 days. Several light regimen schemes were experimented: 23 h light and 1 hour darkness (control group), 8 cycles of 2 h light and 1 h darkness (E1), 6 cycles of 2 h light and 2 h darkness (E2), 12 h light and 12 h darkness (E3). Light regimen shortening resulted in the depression of the daily weight gain and, implicitly, of the body weight, particularly during the second period of growth.

**Keywords:** broiler, light

### ORIGINAL ASPECTS OF THE RESEARCH

Poultry rearing systems (gallinaceous, particularly) evolved towards a marked industrialisation, being characterised by very high stocking rates, the use of two-three tier houses (no natural light), automation of feeding, watering, ventilation and lighting processes.

The increasingly frequent intoxications with dioxin, of food toxic infections and of diseases transmissible to humans (aviary flu) triggered reactions of reevaluating the poultry rearing conditions. Starting from these observations, the European Union developed several regulations that are about to change deeply the rearing and production technologies for gallinaceans.

The technology of broiler rearing stipulates a 23–23.5h light regimen (one or half hour of darkness) with the view of stimulating feed intake and the rate of growth. The short period of darkness is supposed to get the birds accustomed to the lack of light, which might occur due to power circuit failure. The literature (Scheele et al., 1992, Gordon, 1997) mentioned that the mortality rate and the incidence of locomotor apparatus diseases increase in the birds submitted to prolonged light regimens.

It is known that the light radiations influence, by their optical action, the activity of the anterior hypophysis and of the hormonal factors that influence the growth of birds (Scheele et al., 1992). However, broiler exposure to 23h light regimen is an additional factor of stress for the intensive rearing system.

The present paper shows the experimental results of different light regimens and their effect on broiler growth and percentage of viability.

## OBJECTIVE AND METHODS

The experiment used 4000 day-old Cobb broilers reared on the floor on permanent litter, assigned to 4 randomised groups (1 control group and 3 experimental groups). The experimental period was of 49 days.

The broilers had free access to the feed and water. They received standard feeds that provided the nutrient requirement according to the norms.

During the first 7 days all groups had a similar light regimen, i.e. 23 h light and 1-hour darkness. After that period, several light regimen schemes were experimented: 23 h light and 1 hour darkness for the control group (C), 8 cycles of 2 h light and 1 h darkness each for the experimental group 1 (a total of 16 h light and 8 h darkness), 6 cycles of 2 h light and 2 h darkness each for the experimental group 2 (a total of 12 h light and 12 h darkness), 12 h light and 12 h darkness for the experimental group 3. These light regimens were achieved automatically with timers. Light intensity was 10 lx.

The broiler house microclimate was controlled, the environmental parameters being in agreement with the technological recommendations (Technological guidebook, 2000).

The weight progress (weighing on days 1, 21 and 49), the compound feed intake (daily recording by group) and the liveability index were monitored used standard methods.

Broiler performance data were processed statistically with the Student test of significance.

## EXPERIMENTAL DATA AND RESULTS

Body weight at 21 days (Table) was 690 g in group C, 662 g in E1, 642 g in E2 and 614 g in E3. Light regimen shortening to 16 hours (by 8 cycles of 2 h light and 1 h darkness) and to 12 hours (6 cycles of 2 h light and 2 h darkness) caused a significant ( $p \geq 0.05$ ) depression of the body weight. Compared to the control group, body weight depression was 12% ( $p \leq 0.05$ ) in group E3.

As expected, at 49 days, body weight decreased significantly in all experimental groups: by 8% in E1, 10% in E2 and 13% in E3 ( $p \leq 0.05$ ). Light regimen shortening resulted in the depression of the daily weight gain and, implicitly, of the body weight, particularly during the second period of growth. The alternating short cycles of light and darkness periods determined the adaptation of the organism considering that, physiologically, the duration of the intestinal passage is about 3 hours (Burlacu 1985, Stratulat and Marin 1989, Dinu et al 2006).

**Table.** Broiler performance

Item	MU	C	E1	E2	E3
Weight at 21 days	g	690±25.3	662±71.8	642±87.1	614±46.6
	%	100	96	93	90
Weight at 49 days	g	2410±83.3	2217±216.9	2170±225.6	2097±103.8
	%	100	92	90	87
Compound feed intake	kg	3.34±0.24	2.94±0.21	2.90±0.19	2.20±0.22
	%	100	88	87	66
Viability percentage	%	85	88	91	97
Total amount of meat	t	2024.4±117.3	1951.1±126.5	1952.1±134.1	2033.8±105.8
	%	100	96.4	96.4	100.5

Feed intake was significantly influenced by the light regimen shortening, being 12%, 13% ( $p \leq 0.05$ ) and 34% ( $p \leq 0.01$ ) lower in groups E1, E2 and E3, respectively, than in the control group. The shorter light periods determined the birds to crowd at the feeding trough. The alternating light / darkness cycles (groups E1 and E2) caused the birds to fail reach the sensation of satiety because the broilers had to eat the diet in a period as short as possible. Even if the feeding front was provided according to the rearing technology, an accentuation of the social hierarchy (of group) was observed, which generated confrontations between the dominant birds. As a consequence, group heterogeneity increased. The 12 hours light, 12 hours darkness programme (group E3) provided sufficient time for feeding and rest, which met the physiological requirements of the broilers.

The viability percentage (Table) increased from 85% (control) to 97% group E3. As a consequence, the total amount of delivered meat was 2024.4 t in the control group; 1951.7 t in E1; 1952.1 t in E2 and 2033.8 t in E3. The means were statistically equal.

### CONCLUSIONS

1. Concomitantly with the increased concern for the welfare and protection of the poultry reared in intensive systems, new light regimen schemes are sought, which to meet both the physiological and the productive requirements of the birds.
2. The alternating cycles of 2 hours light and 2 hours darkness (E2), starting with the second week of life, did not influence adversely broiler performance.
3. The sudden switch on and off of the light in short cycles (2 h light and 1 h darkness each for the experimental group 1 and 2 h light and 2 h darkness each for the experimental group 2) caused psychic stress to the broilers that may result in behavioural disorders.
4. The 12 h light and 12 h darkness tested for the experimental group 3 provided the highest level of welfare for the broilers as to their physiological requirements of feeding and resting. As a consequence, the viability index increased to 97%, while the total amount of delivered meat was statistically similar to that of the control group.

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## STUDY OF THE THERMOREGULATION CAPACITY OF TRANSYLVANIA NAKED NECK CHICKS COMPARED TO RHODE ISLAND CHICKS

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### SUMMARY

In Romania there is a poultry breed raised in the hill region of Transylvania which has a better thermolysis than other breeds because its body plumage is up to 30% less than the normal one. The study presents the adaptive capacity to heat stress specific to this breed by analysing the growth performance of chicks and some physiological indicators.

**Keywords:** thermoregulation, stress

### MATERIAL AND METHODS

Poultry perceive differently the environmental stimuli as stressors, according to their individual experience on the length, intensity and frequency of the contact with those stimuli, according to the level of phenotypic expression of the interaction between the genotype and the environment. Within this context appears the phenomenon of tolerance to heat that can be a display of the ability some poultry breeds have to increase considerably heat loss through convection and radiation, or it can be a genetic advantage (Yahav et al., 1997, Cooper et al., 1998).

The study monitored the advantage of the lower plumage body by performing a comparative analysis of certain physiological indicators of two poultry populations, Transylvania naked neck chicks and Rhode Island chicks submitted to different environmental temperatures. The value correlations between the growth performance and the physiological indicators of the chicks from these breeds are unique and the product of this working team. The experiment was conducted on two poultry populations, Transylvania naked neck and Rhode Island, reared for egg and meat production in the population households from Transylvania.

The trial involved 72 chicks (males and females) aged 6 weeks, with the average body weight 480 g, assigned to 3 groups of 24 chicks each, housed in 6 cages placed in environmentally controlled rooms. For two weeks the growth program of the chicks was as follows: 12 hours of light per day and standard diet with 17% CP and 2850 Kcal ME. The birds had free access to the feed and water. During this period the chicks were submitted to constant but different environmental temperatures: 22°C and 55% RH for group 1 (control), 15°C and 65% RH for group 2 and 38°C and 45% RH for group 3. Feed ingesta was recorded on a daily basis and the weight gain, average daily gain and feed conversion ratio were determined weekly calculating the average values per group and the standard deviation.

The rectal temperature was measured by inserting a digital thermometer for 6 cm into the rectum. Digital thermometers were also to determine the surface temperature. To that purpose they were introduced subcutaneously through a small incision in the skin of the neck at the level

of the axile in a region not covered by feathers. The recordings were done individually, four times a day and the weekly averages and the standard deviation were calculated for the three groups. Each week blood samples were taken from the brachial vein into syringes on heparin and assayed for hematocrit using the micromethod and for haemoglobin using the photocolometric method. The experimental results were processed with statistics software for the arithmetic mean and standard deviation. The results were used to evaluate the physiological response of the two poultry populations exposed to different environmental temperatures.

## EXPERIMENTAL DATA AND RESULTS

The neuroendocrine mechanisms triggered by the varied action of the different stressors are quite stereotypic: initially, the simpatico-adrenal mechanism, then the hypothalamic–adenohypophysis-corticosuprarenal axis, which are able to maintain or restore homeostasis.

Under an environmental temperature of 15°C, the temperature recorded in Transylvania naked neck chicks at the skin surface in the neck area is significantly higher, by 31%, than the similar temperature recorded in Rhode Island chicks. The difference of temperature recorded at the axile of the two populations was not significant (Table 1). At the environmental temperature of 38°C, the difference of temperature recorded at the skin surface in the neck area was 3.6°C, which means 10.4% higher heat loss in the Transylvania naked neck chicks compared to Rhode Island chicks. The heat loss recorded at the axile of the two populations was not significant. The variation of the rectal temperature between the two populations both for the environmental temperature of 15°C and of 38°C was not significant (Table 1).

**Table 1.** The arithmetic mean and the standard deviation of the average weekly values for the surface temperature recorded at the neck and axile level and of the body temperature in the two populations of chick aged 6 to 8 weeks under different conditions of temperature

Variables	Ambient temperatures (°C)	Transylvania naked neck chicks	Rhode Island chicks
Surface temperature for the neck	22	36,1 ± 2,40	35,7 ± 2,25
	15	34,3 ± 2,84	26,2 ± 1,80
	38	38,2 ± 3,52	34,6 ± 2,85
Surface temperature under the wing	22	39,3 ± 3,60	39,6 ± 4,24
	15	38,4 ± 2,90	38,1 ± 4,13
	38	41,8 ± 3,45	41,5 ± 3,40
Rectal temperature	22	41,7 ± 3,30	41,4 ± 3,52
	15	41,5 ± 3,25	41,2 ± 3,11
	38	42,7 ± 3,17	43,6 ± 3,80

At the environmental temperature of 38°C, the difference of temperature recorded at the skin surface in the neck area was 3.6°C, which means 10.4% higher heat loss in the Transylvania naked neck chicks compared to Rhode Island chicks, while the heat loss recorded at the axile of the two populations was not significant. The variation of the rectal temperature between the two populations both for the environmental temperature of 15°C and of 38°C was not significant (Table 1).

Table 2 shows the values for haematocrit and haemoglobin according to temperature, in both populations.

**Table 2.** The arithmetic mean and the standard deviation of the average weekly values for the haematocrit and haemoglobin in the two populations of chick aged 6 to 8 weeks under different conditions of temperature

Variables	Ambient Temperatures °C	Transylvania naked neck chicks	Rhode Island chicks
haemoglobin (g/dl)	22	10,2 ± 1,14	9,8 ± 0,90
	15	11,6 ± 1,01	10,4 ± 1,02
	38	9,7 ± 0,89	9,0 ± 0,80
haematocrit (%)	22	28,2 ± 2,76	28,4 ± 2,66
	15	35,2 ± 3,48	32,3 ± 3,20
	38	27,8 ± 2,60	26,5 ± 2,58

Haemoglobin and haematocrit values decreased irrespective of the genotype of the studied populations, under the conditions of environmental heat stress, which explains the activation of the thermolysis processes, while the low environmental temperatures increased haemoglobin and haematocrit values, which shows a build up of heat production to maintain homeostasis. At the environmental temperature of 15°C serum haemoglobin was 11.5% higher in Transylvania naked neck chicks than in Rhode Island chicks, while the average value of the haematocrit was 9% higher in Transylvania naked neck chicks than in Rhode Island chicks. No significant differences were noticed between the two chick populations at the environmental temperature of 38°C. Compared to their control groups the depression of the average values for haemoglobin was not significant in the Transylvania naked neck chicks, while the depression was significant, 8.2%, in Rhode Island chicks. The average values for haematocrit in the populations of Transylvania naked neck chicks (control and experimental) decreased insignificantly, while the depression was significant, 6.7%, in Rhode Island chicks.

At the beginning of the experiment, the average body weight of the 6 weeks-old chicks was 480 g, while after two weeks the chicks raised under normal environmental temperature reached an average body weight of 685 g. When exposed to 15°C the two populations of chicks aged 8 weeks did not display significant differences in the body weight, while at 38°C body weight was 8.8% lower in Rhode Island chicks (compared to the control group reared at 22°C) and 6% lower in Transylvania naked neck chicks (Table 3).

**Table 3.** The arithmetic mean and the standard deviation of the average weekly values for the performance of the two populations of chick aged 6 to 8 weeks under different conditions of temperature

Variables	Ambient Temperatures °C	Transylvania naked neck chicks	Rhode Island chicks
Body weight at the age of 8 weeks (g)	22	688 ± 64,6	682 ± 62,3
	15	684 ± 58,4	678 ± 66,2
	38	647 ± 60,2	622 ± 60,4
Weight gain at the age of 6 to 8 weeks (g/14zile)	22	15,07 ± 1,30	14,42 ± 1,38
	15	14,78 ± 1,20	14,14 ± 1,05
	38	12,50 ± 1,10	10,10 ± 0,09
Food intake N.C. at the age of 6 to 8 weeks (g/zi)	22	45 ± 4,30	42 ± 3,80
	15	58 ± 4,60	49 ± 4,40
	38	41 ± 4,10	36 ± 3,80

The average daily gain achieved at the environmental temperature of 15°C decreased insignificantly in both breeds, but at 38°C, the depression was 17% in Transylvania naked neck chicks of the experimental group compared to the control group and in excess of 30% in the Rhode Island chicks of the experimental group compared to the control group. Compound feed intake increased at the environmental temperature of 15°C by 28.8% in Transylvania naked neck chicks and by 16.6% in Rhode Island chicks, while at 38°C feed intake decreased by 8.8% Transylvania naked neck chicks and by 14.3% in Rhode Island chicks. Under the heat stress, the voluntary feed intake decreases proportionally with the increment of the temperature, of the duration of exposure and with the bird age, ranging from 8 to 25% (Monica Parvu et al., 2006).

At the environmental temperature of 15°C the loss of heat through radiation at the surface of the neck skin was higher in the Transylvania naked neck chicks than in the Rhode Island chicks, which required the birds to intensify their metabolic processes which means higher oxygen consumption for the cellular oxidation of the fatty acids and to stimulate new production of glucose in the liver. The red blood cells from the storage organs were mobilised in response to the increased requirement of oxygen, which increased the haematocrit and the haemoglobin, a process that was done better in the Transylvania naked neck chicks than in the Rhode Island chicks.

The increased heat production under lower environmental temperatures is also supported by a higher feed intake, by the required digestive work and by the specific dynamic action of the feeds. The intensification of cellular energy production was triggered by the activation of the simpatico-adrenal system done by a protein nutritive support. Under the conditions of similar dietary protein levels feed intake increased more in the Transylvania naked neck chicks than in the Rhode Island chicks, which shows a better adaptation to the lower environmental temperatures.

Reducing energy metabolism through a lower feed intake and through cellular biochemical modifications did the adaptation to higher environmental temperatures. The existence of a naked area on the neck intensified heat dissipation through radiation in the Transylvania naked neck chicks compared to the Rhode Island chicks.

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## CONCLUSIONS

1. The higher adaptative capacity of the Transylvania naked neck chicks is the result of a better functional correlation of the activity of the hypophysis, thyroid and adrenal glands, all of them coordinated by the hypothalamus.
2. The particular rusticity of the Transylvania naked neck chicks reared in the area of Transylvania is shown by its ability to do thermoregulation under variable environmental conditions: at higher temperatures they have the advantage of a lower plumage on the breast and abdomen, with completely naked neck up under the gizzard, while they also display resistance to lower temperatures and diseases.

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## RESPONSE OF SUMMER STRESSED GROWING RABBITS TO SOME DIETARY GROWTH PROMOTERS

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### ABSTRACT

An experiment was carried out to investigate the response to some growth promoters as safe alternatives to antibiotics on some performance aspects of rabbits subjected to environmental stress of summer season (29–33°C) and 48–63% relative humidity. Seventy, 6 weeks old-NZW rabbits were equally sexed and allotted to evaluate one of the following seven feed additives: 1: no supplement, 2: 0.5% acetic acid, 3: 0.5% lactic acid, 4: 0.5% a herb mixture of equal parts of sage+oregano+sweet basal, 5: 1.0% of the previous herb mixture, 6: 1.0 g GOS<sup>®</sup> (gluco-oligosaccharides; a prebiotic)/ kg diet, and 7: 1.0 g Bio-Mos<sup>®</sup> (mannan-oligosaccharides; a prebiotic)/ kg diet. The growth trial lasted up to the 13<sup>th</sup> week of age. Results reveal that total weight gain ( $p<0.01$ ), and feed conversion ratio ( $p<0.05$ ) were improved, while a significant decreasing effect ( $p<0.05$ ) in both plasma total lipids and cholesterol with supplements under studied was detected. 0.5% acetic acid, or 1.0% herb mixture decreased ( $p<0.01$ ) the abdominal fat content of the carcass. Histological study indicated that villi height and crypt depth, both ( $p<0.01$ ) were increased with studied additives. pH of blood, cecum and ileum, besides, cecal content of volatile fatty acids, all were not affected by supplements. Conclusively, it is advisable to fortify rabbit diets reared under elevated ambient temperatures with one of the suggested additives, especially, with the prebiotics GOS<sup>®</sup>, Bio-Mos<sup>®</sup> or the herb mixture at 0.5% supplementation level to improve growth and health of rabbits grown under high ambient temperature conditions.

**Keywords:** rabbit, summer season, performance, organic acids, medicinal plants, and prebiotics

### INTRODUCTION

High ambient temperature coincided with elevated relative humidity and digestive disorders around weaning are the most limiting factors in rabbit production in the tropics and subtropics. Therefore, feed additives which enhance performance under these critical conditions are of great interest for nutritionists and stockman (Abdel-Khalek., 2003 and Fonseca *et al.*, 2004). Recent evidence on the development of resistance in zoonotic organisms of animal origin and consumers' claims for safety animal foods urged the necessity for other natural, safe, reliable, and economic additives that serve the same goals achieved by antibiotics.

Organic acids, besides its anti-biotic like action, through inhibiting action for the intestinal bacteria competing with the host for available nutrients (Hyden, 2000), serve as substrates in the intermediary metabolites, increase gastric proteolysis and availability of some elements that complex with (Kirchgessner and Roth, 1988). Upon rabbit weaning, a predisposing factor to diarrhea is reduced acidity (a more alkaline pH) of the cecal contents, administrating a source of acid increased the cecum acidity and may reduce enteritis incidence (Morisse *et al.*, 1985). It

seems that when evaluating the effect of organic on rabbit performance, hardly any trends could be demonstrated. On one hand, El-Kerdawy (1996) and Scapinello *et al.*, (1998) reported encouraging results when evaluating different sources of organic acids in rabbit diets; on the other hand, El-Allawy (2001) reported no advantage to supplement organic acids to rabbit.

Essential oils from herbs have anti-microbial and anti-oxidant activities also have hypo-cholesterolemic effects, indeed, a growth enhancing effect has been reported (Lee *et al.*, 2004). Sage extracts contain active antioxidative factors such as phenolic diterpenes, flavonoids and phenolic acids (Ho *et al.*, 2000). Terpenoids in the dried green leaves in sage (*Salvia officinalis* L.) were regarded as the most promising sources of natural anti-oxidants, where sage protected against H<sub>2</sub>O<sub>2</sub>-induced DNA damage (Aheren *et al.*, 2007) and play an important role in protecting mitochondria function against various oxidative stress (cited from Ibrahim *et al.*, 2002). When incorporated at a range of 0.25–1.0% in growing rabbit diets, increased live weight gains, improved feed conversion ratio, but fluctuated plasma values of total protein, total lipids and cholesterol were reported (Ibrahim *et al.*, 2002 and Khayyal, 2006). The main components in oregano-oil (*Origanum vulgare* L.), are carvacrol (60–80%) and thymol (2%); carvacrol inhibits the metabolism of the microbial cell wall (Smink, 2000). Indeed, has a significant antioxidant effect (Botsoglou *et al.*, 2004), among several hundred of plants, Blomhoff (2004) found that oregano, followed by sage were ranked first and second to have anti-oxidant activity against oxidative stress. Also, oregano has a growth promoting and a hypolipidemia action (Ibrahim *et al.*, 2000). Sweet basil (*Ocimum basilicum* L.) with its high content of anethole, showed anti-microbial activity (Lachowicz *et al.*, 1998), also, the phenolic compounds such as rosmarinic acid in sweet basil has anti-oxidant activity (Jayasinghe *et al.*, 2003). and has a clear role in enhancing growth performance and lowering blood content of total lipids and cholesterol (Ibrahim *et al.*, 2000). It is considered beneficial to use a mixture of different sources of medicinal plants rather than a single source (Moleyar and Narasimham, 1992), the synergistic properties of different oils can keep the effective anti-microbial concentration of essential oils as low as possible due to characteristic flavors (Lee *et al.*, 2004), thus improving the anti-microbial activity in spite of low dosages.

Oligosaccharides as prebiotics are a class of carbohydrates that are not absorbed or digested in the small intestine of animals, but readily fermented by the intestinal microflora. This may result in changes in this flora, thereby increasing the number of beneficial micro-organisms, while repressing the harmful bacteria (Quigley, 2004). The phosphorylated mannan oligosaccharides, Bio-Mos<sup>®</sup>, derived from the outer cell wall of the yeast *Saccharomyces serivisae*, consist of a mannan component. The structure of the mannan resembles that of the carbohydrates on the animal gut wall. In theory, pathogenic, growth inhibiting microbes that normally adhere to mannans on the gut wall may instead bind to the mannan component of Bio-Mos<sup>®</sup>, so these pathogens flushed out from the upper gut, and do not attach to the mucosal receptors. Elimination of the pathogens would presumably enhance the health and growth of the animal. While, other sources of oligo-saccharides, is gluco-oligosaccharides act as substrates for 'desired' micro-organisms (Newman, 1994 and Huyghebaert, 2003). Providing Bio-Mos<sup>®</sup>, resulted in reduced mortality rate, improved feed conversion ratio (FCR) and similar daily weight gains (DWG) compared to oxytetracycline (Fonseca *et al.*, 2004). Both MOS and oxytetracycline induced longer villi, increased absorption area and caecal VFA's, moreover, decreased caecal pH compared to the control not medicated (Pinheiro *et al.*, 2004), while, Gidenne (1995) found no effect on DWG, FCR and caecal VFA's pattern when offered GOS<sup>®</sup> to rabbits fed low crude fiber diet.

The objective of this study was to evaluate the response of growing rabbits to some growth promoters as save alternatives to antibiotics.

## MATERIAL & METHODS

The study was carried out during August-September months, at Animal Prod. Res. Inst. Station, ARC, Egypt. Seventy-6 week old New Zealand White rabbits were evenly sexed, weighed and individually caged to evaluate one of the following seven feed additives: 1: no supplement, 2: 0.5% acetic acid (96%), 3: 0.5% lactic acid (90%), 4: 0.5% a herb mixture of equal parts of sage+oregano+sweet basil, 5: 1.0% of the previous herb mixture, 6: 1.0 g GOS<sup>®</sup> (prebiotic)/ kg diet, and 7: 1.0 g Bio-Mos<sup>®</sup> (prebiotic)/ kg diet. The herb mixture included an equal parts of sage + oregano + sweet basil. GOS<sup>®</sup> is a glucan-oligosaccharides, while, Bio-Mos<sup>®</sup> is a mannan oligosaccharide, both are products of Alltech Inc., USA. Additives studied were supplemented on the expense of total diet. Basal diet was formulated according to the NRC (1977) recommendation for growing rabbits. Ingredient and chemical composition of the basal diet are presented in Table (1). Total essential oils of each medicinal plant incorporated were conducted (Guenther, 1961). Rabbits were kept under managerial routine. Rabbitry air temperature and relative humidity were recorded daily. Growth trial lasted for 7 weeks, after which 3 rabbits of each treatment were fasted for 12 hours, and then slaughtered to complete bleeding. Blood plasma samples were collected for further determination of total protein, albumin, total lipids and total cholesterol. While, plasma globulin was deduced as the difference between total protein and albumin. Ileum samples were collected for histological study, absorption area was calculated (Fonseca *et al.*, 2004) as [villi height/ (villi height/crept depth)]. Volatile fatty acids (acetic, propionic and butyric) of the cecal content (HPLC; Kenaur-model) were determined. On slaughtering, pH of blood, cecum, and ileum (pH digital-pH meter) were measured. Hot carcass, giblets (heart+liver+kidneys+lungs), total edible parts (hot carcass+giblets+head) and abdominal fat percentages were proportioned to live weight of rabbits assigned for slaughter test. Data were subjected to a one-way analysis using SAS (1990). Variables having significant differences were compared using Duncan's Multiple Range Test (Steel and Torrie, 1960).

**Table 1.** Ingredients and calculated chemical composition of the basal experimental diet

Ingredients	%	Calculated chemical composition
Wheat bran	24.45	DE 2651 (kcal.kg <sup>-1</sup> diet)
Barley	22.50	CP 17.09%
Soybean meal (44% CP)	19.25	CF 12.03%
Clover straw	17.80	Calcium 0.81%
Yellow corn	9.50	Phosphorus 0.54%
Molasses	4.00	Lysine 0.80%
Limestone	1.77	Methionine and Cystine 0.61%
Sodium chloride	0.30	
Vitamins and minerals mixture *	0.30	
DL-Methionine	0.13	
Total	100.0	

\*Supplied per kg. of diet: 12000 IU vit.A; 2200 IU vit. D<sub>3</sub>; 10 mg vit. E; 2.0 mg vit. K<sub>3</sub>; 1.0 mg vit. B<sub>1</sub>; 4.0 mg vit. B<sub>2</sub>; 1.5 mg vit. B<sub>6</sub>; 0.0010 mg vit. B<sub>12</sub>; 6.7 mg vit. PP; 6.67 mg vit. B<sub>5</sub>; 0.07 mg B<sub>8</sub>; 1.67 mg B<sub>9</sub>; 400 mg Choline chloride; 133.4 mg Mg; 25.0 mg Fe; 22.3 mg Zn; 10.0 mg Mn; 1.67 mg Cu; 0.25 mg I and 0.033 mg Se.

## RESULTS AND DISCUSSION

Throughout the seven-week growth trial, the recorded daily air temperatures were laid between (29–33°C), while relative humidity values were ranged between 50–63%. The total essential oils in oregano was 1.25%, in sage was 1.15%, while in sweet basil was 0.80%

### 1 – Growth performance

Table (2) illustrates the results growth performance criteria; initial live weight, total weight gain, feed intake and feed conversion ratio. It is clear that all suggested additives significantly ( $p < 0.001$ ) surpassed the control in total weight gain, especially with 0.5% herb mixture supplementation, also improved feed conversion ratio ( $p < 0.005$ ) was recorded. These results and those reported by Scapinello *et al.*, (1998) agree that a reduction in feed intake and improved in feed conversion ratio were attained when rabbits fed on diets with an incremental level of 0.5% of acetic acid up to 2.0%. Also, El-kerdawy (1996) found that fumeric, citric, and malic acids at the rated of 0.5% increased weight gain and return of rabbits. While, El-Allawy (2001) found that citric acid (5.0 g/kg diet) had no effect on rabbit weight gain, but decreased feed intake, moreover, worsened feed conversion ratio. It is worth noting that Tsiloyiannis *et al.*, (2001) found that lactic acid was the most effective organic acid to improve performance traits of pigs. This improvement in growth rate with organic acids in this study may be due to decreased pH of the cecum and ileum to levels not favorable for pathogens, also that it serves as substrates in the intermediary metabolites, increase gastric proteolysis and availability of some elements that complex with (Kirchgeßner and Roth, 1988). Regarding the herb mixture, Ibrahim *et al.*, (2000) using sweet basil or oregano at the level of 0.5%, and again, Ibrahim *et al.*, (2002) using 0.5 or 1.0% sage found that such supplements significantly increased weight gain, feed intake, and improved feed conversion of rabbits. Khayyal (2006) found that 0.25 vs. 0.0, 0.5 or 1.0% of dried sage leaves was more effective in enhancing growth performance of rabbits. While, Botsoglou *et al.*, (2004) reported that dietary oregano essential oil (100 or 200 mg/kg diet) exerted no growth promoting effect on rabbits. It is obvious that the lower level of herb mixture (0.5%) gave the highest weight gain, and the highest absorption area value (Table 4), also compared with the higher level (1.0%) of herb mixture it gave higher weight gain and feed intake, in this regard, Lee *et al.*, (2004) considered that it is beneficial to keep the effective anti-microbial concentration of essential oils as low as possible due to characteristic flavors. Also the improvement with using a mixture may be due to synergistic properties of different oils (Moleyar and Narasimham (1992). Respecting prebiotics, our results conflict with those of Gidenne (1995) who reported no effect on weight gain or feed conversion when rabbits were offered GOS<sup>®</sup> to low crude fiber diet, but agree with Fonseca *et al.*, (2004) who found that providing Bio-Mos<sup>®</sup>, resulted in improved feed conversion ratio (FCR) and similar daily weight gains (DWG) compared to oxytetracycline. This may be attributed to that Bio-Mos<sup>®</sup> induced longer villi and increased absorption area compared to the control not medicated (Pinheiro *et al.*, 2004).

**Table 2.** Effect of studied feed supplements on growth performance

Variable Treatment	Initial live weight (g)	Total weight gain (g)	Feed intake (g)	Feed conversion ratio
Control	619±18	1128 <sup>c</sup> ±22	4656±171	4.14 <sup>b</sup> ±0.16
Acetic acid	617±21	1202 <sup>b</sup> ±23	4459±111	3.71 <sup>a</sup> ±0.05
Lactic acid	620±18	1224 <sup>ab</sup> ±22	4564±88	3.73 <sup>a</sup> ±0.04
Herb mix (0.5%)	621±18	1279 <sup>a</sup> ±18	4843±145	3.79 <sup>a</sup> ±0.11
Herb mix (1.0%)	616±19	1207 <sup>ab</sup> ±30	4517±155	3.74 <sup>a</sup> ±0.09
GOS <sup>®</sup>	619±24	1267 <sup>ab</sup> ±21	4790±66	3.78 <sup>a</sup> ±0.02
Bio-Mos <sup>®</sup>	619±20	1224 <sup>ab</sup> ±26	4703±158	3.83 <sup>a</sup> ±0.06
Significance	ns	**	ns	*

Means in the same column within each factor differently superscripted are significantly different. ns: not significant, \* : ( $p < 0.05$ ), and \*\*: ( $p < 0.01$ ),

## 2 – Plasma constituents

Results in Table (3) indicate that of the studied plasma constituents, only plasma total lipids and cholesterol were significantly affected by supplements evaluated. It is clear that 1.0% herb mixture and GOS<sup>®</sup> recorded the lowest total lipids and cholesterol, respectively. In this respect, Ibrahim *et al.*, (2000) found that sweet basil or oregano at the level of 0.5%, each significantly had a hypo-lipidemic effect. Also, the decrease in cholesterol level with increasing the level herb mixture may be due to that pure components of essential oils inhibit hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity (Crowell, 1999), Which is a key regulatory enzyme in cholesterol synthesis, yet, Khayyal, (2006) reported a slight decrease in rabbits' plasma total lipids and cholesterol when fed on diets fortified with sage leaves at levels ranged between 0.25 and 1.0% in an incremental level of 0.25%. With prebiotic supplementation, Kannan *et al.*, (2005) reviewed that the decreased in blood cholesterol with prebiotic supplementation is a result to the cholesterol assimilation by *Lactobacillus*, as the prebiotic supplementation could enhance the lactobacilli count. *Lactobacillus spp.* are able to incorporate cholesterol into the cellular membrane of the organism. The decrease in plasma total lipids and cholesterol with organic acid supplementation may be due to the inhibition of lipogenesis in liver as reported by Fushimi *et al.*, (2006). Also results agree with El-kerdawy (1996) who found that plasma total lipids decreased, while total protein was not affected by fumeric, malic and citric acids supplementation at the rated of 0.5% of rabbit diets.

**Table 3.** Effect of studied feed supplements on some plasma constituents

Variable Treatment	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Total lipids (mg/dl)	Cholesterol (mg/dl)
Control	6.81±0.20	5.02±0.48	1.79±0.37	488 <sup>ab</sup> ±14	104 <sup>a</sup> ±4.0
Acetic acid	7.69±0.51	4.72±0.36	2.97±0.26	456 <sup>abc</sup> ±28	91 <sup>b</sup> ±2.3
Lactic acid	7.77±0.42	5.05±0.05	2.72±0.37	406 <sup>abc</sup> ±19	93 <sup>ab</sup> ±4.0
Herb mix (0.5%)	7.18±0.41	5.21±0.28	1.97±0.12	384 <sup>bc</sup> ±17	95 <sup>ab</sup> ±1.2
Herb mix (1.0%)	7.90±0.29	5.14±0.38	2.75±0.65	360 <sup>c</sup> ±53	90 <sup>b</sup> ±5.4
GOS <sup>®</sup>	6.92±0.16	4.43±0.22	2.49±0.30	478 <sup>ab</sup> ±38	72 <sup>c</sup> ±3.5
Bio-Mos <sup>®</sup>	7.20±0.30	4.61±0.16	2.59±0.34	508 <sup>a</sup> ±34	95 <sup>ab</sup> ±3.6
Significance	ns	ns	ns	*	*

ns: not significant,

\*: means in the same column within each factor differently superscripted are significantly different ( $p < 0.05$ )

### 3 – Villi measurements and absorption area

Results in Table (4) reveal that herb mixture at the level of 0.5%, followed by both prebiotic sources increased ( $p<0.01$ ), both villi height and crept depth, and absorption area ( $p=0.06$ ) compared with control. In this connection, Pinheiro *et al.*, (2004) reported that Bio-Mos<sup>®</sup> at the rate of 1.0, 1.5 and 2.0 kg/ton rabbit diet resulted in a significant increase in villi height, which in turn translated into a numeric increased absorption area of the intestine over that reported for the control group. While, Mourão *et al.*, (2004) reported that fructo-oligosaccharides had no effect on villi measurements when added to rabbit diet. Elimination of the pathogens would presumably enhance the health and of the gut condition as reported by (Newman, 1994 and Huyghebaert, 2003).

**Table 4.** Effect of studied feed supplements on villi measurements ( $\mu\text{m}$ ) and absorption area ( $10^3 \mu \text{m}^2$ )

Variable Treatment	Villi height	Crept depth	Villus height Crept depth	Absorption Area
Control	635 <sup>c</sup> ±10	160 <sup>c</sup> ±3.9	3.98±0.07	166.3±4.19
Acetic acid	650 <sup>c</sup> ±13	172 <sup>bc</sup> ±5.9	3.82±0.11	168.8±7.89
Lactic acid	665 <sup>bc</sup> ±19	167 <sup>bc</sup> ±8.0	4.04±0.12	163.8±8.85
Herb mix (0.5%)	740 <sup>a</sup> ±21	203 <sup>a</sup> ±7.9	3.72±0.19	198.8±11.1
Herb mix (1.0%)	689 <sup>abc</sup> ±26	187 <sup>abc</sup> ±12.1	3.79±0.18	183.8±12.1
GOS <sup>®</sup>	724 <sup>ab</sup> ±23	192 <sup>ab</sup> ±10.9	3.85±0.14	183.8±8.65
Bio-Mos <sup>®</sup>	721 <sup>ab</sup> ±25	193 <sup>ab</sup> ±11.3	3.82±0.17	197.5±13.6
Significance	**	**	ns	ns

ns: not significant,

\*\* : means in the same column within each factor differently superscripted are significantly different ( $p<0.01$ )

### 4 – Cecal volatile fatty acids concentration and pH of cecum, ileum, and blood

Results in Table (5) indicate that, any of the studied supplements had a significant effect on volatile fatty acids production (acetic, propionic and butyric) or pH of the cecum, ileum or blood ( $p=0.06$ ). El-Allawy (2001) found that citric acid (5.0 g/kg diet) had no effect on rabbit cecum pH. Mourão *et al.*, (2004) reported that fructo-oligosaccharides had no effect on VFA production when added to rabbit diet (0.36g/kg diet). While, Pinheiro *et al.*, (2004) reported that Bio-Mos<sup>®</sup> at the rate of 1.0, 1.5 and 2.0 kg/ton rabbit diet resulted in a significant increase in VFA's and a significant decrease in cecal pH. It is worth noting that an important tool to qualitatively evaluate the microbial activity in the intestine is to measure the volatile fatty acids concentrations as end-products of microbial fermentation (Bellier and Gidenne, 1996 and Pinheiro *et al.*, 2004). Also, that the rabbit is unusual in that butyric acid is a more important VFA than propionic acid as an end product of cecal fermentation, this may be important in preventing enteritis (Cheeke, 1987).

**Table 5.** Effect of studied feed supplements on cecal volatile fatty acids concentration (mmol.1000 ml<sup>-1</sup>) and pH of cecum, ileum, and blood

Variable Treatment	Acetic acid	Propionic acid	Butyric Acid	pH cecum	PH ileum	pH blood
Control	46.5±0.6	21.9±2.3	29.7±1.6	6.17±0.07	7.36±0.03	7.32±0.06
Acetic acid	45.2±2.0	22.2±0.9	28.4±0.5	6.05±0.16	7.24±0.05	7.39±0.05
Lactic acid	48.4±0.8	20.0±0.1	27.4±1.0	5.97±0.11	7.31±0.09	7.50±0.02
Herb mix (0.5%)	47.8±0.2	22.9±0.7	27.2±1.9	6.35±0.15	7.27±0.06	7.39±0.10
Herb mix (1.0%)	46.7±2.8	25.0±3.4	25.9±2.1	6.26±0.09	7.35±0.15	7.56±0.02
GOS <sup>®</sup>	47.8±0.9	23.5±0.2	26.0±0.8	6.20±0.22	7.38±0.18	7.50±0.03
Bio-Mos <sup>®</sup>	44.3±1.3	22.1±3.0	29.6±1.5	6.30±0.18	7.48±0.07	7.49±0.02
Significance	ns					

ns: not significant

### 5 – Carcass characteristics of rabbits

Results in Table 6 indicate that relative to the slaughter weight, hot carcass, giblets, and total edible parts percentage, were not significantly affected by supplements under study, while, abdominal fat % was significantly decreased as summer reared rabbit diets were fortified with acetic acid or the 1.0% herb mixture. El-Allawy (2001) found that citric acid (5.0 g/kg diet) had no effect on rabbit dressing %. Ibrahim *et al.*, (2000) found that sweet basil or oregano at the level of 0.5% increased significantly dressing and giblets %. Khayyal (2006) reported higher total edible parts of rabbit carcass % as sage leaves were included in the diet at 0.25% compared to the control or those supplemented with 0.50 or 1.0%. Mourão *et al.*, (2004) reported that fructo-oligosaccharides had no effect on weights of liver when added to rabbit diet (0.36g/kg diet).

**Table 6.** Effect of studied feed supplements on some carcass characteristics (%)

Variable Treatment	Slaughter weight (g)	Hot carcass	Giblets	Total edible	Abdominal fat
Control	1778±53	50.9±1.2	4.5±0.6	61.2±1.5	0.72 <sup>a</sup> ±0.04
Acetic acid	1884±39	52.3±0.8	4.8±0.2	62.7±0.7	0.46 <sup>b</sup> ±0.07
Lactic acid	1886±49	51.2±1.6	4.6±0.1	61.2±1.6	0.61 <sup>a</sup> ±0.04
Herb mix (0.5%)	1921±38	52.4±1.2	4.6±0.2	62.5±1.5	0.63 <sup>a</sup> ±0.04
Herb mix (1.0%)	1829±15	52.4±0.6	5.3±0.3	63.3±0.3	0.46 <sup>b</sup> ±0.03
GOS <sup>®</sup>	1838±24	52.5±0.3	4.9±0.2	63.2±0.1	0.63 <sup>a</sup> ±0.02
Bio-Mos <sup>®</sup>	1900±30	50.2±0.3	4.6±0.6	60.2±0.5	0.71 <sup>a</sup> ±0.03
Significance	Ns	ns	ns	ns	**

ns: not significant,

\*\* : Means in the same column within each factor differently superscripted are significantly different ( $p < 0.01$ )

## CONCLUSION

The current study proved that these additives were effective to correct some of deleterious effects of high ambient temperatures on rabbit growth performance and some biological functions.

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## EFFECT OF RESTRICTED ACCESS TO WATER ON BROILER HOUSE CLIMATE AND PRODUCTIVITY OF BROILER CHICKENS

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### SUMMARY

The aim of the experiment was to determine the effect of restricted access to water for 8 h/day on broiler house climate and productive results of broiler chickens.

The results obtained showed that restricting access to water for 8 h/day reduced litter and manure moisture compared to the control group, leading to lower ammonia levels in the air. The use of restricted access to water had no adverse effect on the final body weight of the chickens, feed conversion or results of carcass analysis.

It is concluded that restricting access to water for 8 h/day improves the climatic conditions of the broiler house without any significant effect on broiler performance.

**Keywords:** restricted access to water, broiler house climate, productivity, broiler chickens

### INTRODUCTION

The high productivity of birds is conditional on an adequate supply of drinking water. It is involved in all life processes and accounts for 50–70% of the bird's total body weight (Chołocińska et al., 1997). According to North and Bell (1990), the daily amount of drinking water supplied to a broiler house should range from 0.3 to 0.5 l/bird. A higher demand for water is due to higher water consumption depending on bird's age, weight, sex, health, feed intake and composition, and type and technical condition of drinkers (Chołocińska et al., 1997; Miller et al., 1988; Bessei et al., 1999).

Under appropriate climatic conditions, high water intake is undesirable because of excessive respiratory evaporation and fecal and urinary excretion of water (Bennett and Leeson, 1989). In turn, excessive moisture in livestock buildings reduces the quality of litter and climate, especially by increasing the ammonia concentration in air (Chołocińska et al., 1997; Bessei et al., 1999; Sosnówka-Czajka et al., 2004). Many authors believe that increased levels of ammonia in livestock buildings influence the body's physiological processes and thus negatively affect production results and the quality of poultry products obtained (Kristensen and Wathes, 2000; Al-Homidan et al., 2003).

According to Chołocińska (1998), not only the birds' basic life processes but also their productivity can be regulated by the amount of water supplied.

Therefore, the aim of the experiment was to determine the effect of restricting access to water for 8 h per day on broiler house climate and productive results of broiler chickens.

## MATERIAL AND METHODS

A total of 8000 day-old Hubbard chicks were assigned to 2 groups. Each group had 20 subgroups with a stocking density of 15 birds/m<sup>2</sup>.

In group I (control), birds had free access to water throughout the experiment, and in group II (experimental), from 21 days of rearing, birds were given water for 16 h/day at 0900-1200, 1400-1700 and 2000-0600 h. Chickens were reared on litter to 42 days of age and fed *ad libitum* with standard diets.

During the experiment, individual body weight of birds, feed intake, water intake and mortality were monitored every week. At 21, 28, 35 and 42 days of rearing, air concentrations of NH<sub>3</sub> were measured using Dräger tubes at four points diagonally through the broiler house, 30 cm above litter. At 21, 28, 35 and 42 days of rearing, litter moisture and fecal dry matter content were also determined. On the last day of rearing, 20 birds with close to average body weight were selected from each group. After slaughter and cooling, they were subjected to a simplified carcass analysis.

The results were analysed statistically by way of one-way analysis of variance using Statgraphics plus 6.0.

## RESULTS

A deterioration in the broiler house environment is associated with litter moisture and increased ammonia concentration in the air (Sosnowka-Czajka et al., 2004). In our study, restricting access to water for 8 h/day from 21 days of rearing significantly reduced ammonia concentration in the air at 28 and 35 days, and highly significantly on day 42 of the experiment (Tab. 1). The reduced level of ammonia in the air in the experimental group was associated with a greater dry matter content of litter ( $p < 0.01$ ) and a greater dry matter content of manure ( $p < 0.05$ ) during the final weeks of rearing (Tab. 2 and 3). Bessei et al. (1999) reported that restricting water intake by limiting water supply or the duration of access to water improved manure consistency, while according to Herbut (1997), ammonia volatilization from litter can be considerably reduced by rapid drying of excreta.

In the present study, restricting access to water did not reduce the final body weight of the birds (Tab. 4), despite the fact that at 21 and 35 days of rearing, chickens from the experimental group were characterized by a significantly lower body weight compared to the control birds. In the last week of the experiment, compensatory growth occurred in birds from the experimental group and at 42 days of rearing the body weight of birds from both groups was 2439 g. Similar results were obtained by Chołocińska (1988), who exposed broiler chickens to 12-, 18- and 24-hour water deprivation from 1 to 3 weeks of age. Likewise, Gerry (1980), Bennett and Leeson (1989) and Chamblee et al. (1989) did not find any decreases in birds' body weight after restricting access to water. However, Ross (1960) reported that both body weight and feed intake decreased in birds that had daily 30-minute access to water over a 6-week period of rearing.

According to the literature, there is a close relationship between the amount of water consumed and feed intake (Chołocińska, 1988; Miller et al., 1988; Ross et al., 1981). In our study, we observed slightly lower feed intake in birds from the experimental group, paralleled by a higher water intake per kg weight gain, but these differences were not significant (Tab. 5). A non-significant effect of water deprivation on feed intake by birds was also reported by Chołocińska (1988) and Miller et al. (1988). On the other hand, Watkins and Novilla (1994) found that

restricted access to feed and water caused a highly significant decrease in feed intake, weight gains and feed conversion, but unlike in our study, they failed to observe the effect of the experimental factor on the health of birds (Tab. 5).

Skomorucha et al. (2006) reported that restricted access of broiler chickens to water adversely affects meat quality. In our study, we did not find statistically significant differences between the groups in the results of carcass analysis (Tab. 6), although there was a tendency towards slightly higher dressing percentage and greater content of breast and leg muscles in birds from the experimental group. Birds from group II, in which water was restricted, were also characterized by a lower content of leg bones, abdominal fat and giblets.

## CONCLUSION

The results obtained showed that restricting access to water for 8 h/day reduced litter and manure moisture compared to the control group, leading to lower ammonia levels in the air. Water deprivation had no adverse effect on the final body weight of the chickens, feed conversion or results of carcass analysis. It is therefore concluded that restricting access to water for 8 h/day improves the climatic conditions of the broiler house without any significant effect on broiler performance.

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**Table 1.** Ammonia concentration in air (ppm)

Day of rearing	Group	
	1	2
21	4.50 ± 0.50	2.75 ± 0.75
28	8.50 ± 1.19 a	4.00 ± 1.00 b
35	9.00 ± 1.35 a	5.25 ± 0.25 b
42	21.25 ± 1.25 A	10.00 ± 0.00 B

A,B – values in rows marked with different letters differ highly significantly  
a,b – values in rows marked with different letters differ significantly

**Table 2.** Litter dry matter (%)

Day of rearing	Group	
	1	2
21	70.39 ± 1.65 A	58.42 ± 1.17 B
28	67.95 ± 0.92	64.57 ± 1.89
35	61.47 ± 1.73 A	73.14 ± 1.65 B
42	62.23 ± 1.60 A	71.34 ± 1.03 B

A,B – values in rows marked with different letters differ highly significantly

**Table 3.** Dry matter content of manure (%)

Day of rearing	Group	
	1	2
21	21.11 ± 0.61	22.15 ± 0.88
28	21.05 ± 0.27 a	22.42 ± 0.33 b
35	21.65 ± 0.34 a	23.73 ± 0.65 b
42	21.92 ± 0.24 a	23.33 ± 0.44 b

a,b – values in rows marked with different letters differ significantly

**Table 4.** Body weight of broiler chickens (g)

Day of rearing	Group	
	1	2
7	155.25 ± 1.43	151.32 ± 1.61
14	401.75 ± 8.04	398.00 ± 3.64
21	801.50 ± 7.53 a	776.25 ± 6.59 b
28	1356.98 ± 10.01	1349.95 ± 9.17
35	1980.76 ± 16.88 a	1915.00 ± 19.16 b
42	2439.17 ± 26.84	2439.53 ± 28.56

a,b – values in rows marked with different letters differ significantly

**Table 5.** Feed and water conversion and broiler chicken mortality from 1 to 42 days of rearing

Item	Group	
	1	2
Feed conversion (kg/kg weight gain)	1.92	1.88
Water conversion (l/kg weight gain)	3.48	3.50
Mortality (%)	6.17 ± 0.91	7.12 ± 0.91

**Table 6.** Results of carcass analysis of 42-day-old broiler chickens

Item	Group	
	I	II
%		
Dressing percentage:		
• with giblets	77.22±0.52	78.06±0.28
• without giblets	73.87±0.46	74.27±0.30
Muscles:		
• breast	23.99±0.34	24.56±0.26
• leg	18.96±0.28	19.11±0.30
Leg bones	5.32±0.12	5.29±0.15
Abdominal fat	2.20±0.12	2.17±0.22
Liver	2.79±0.12	2.60±0.08
Giblets	4.47±0.13	4.38±0.08



Tuesday, June 19, 2007

## **PLENARY LECTURES**





## HEALTH AND WELFARE IN GENETICALLY HIGH PRODUCING DAIRY COWS AND ITS ECONOMICAL IMPLICATIONS

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### SUMMARY

In many countries the milk production per cow has more than doubled in the last 40 years. The increase in production has been accompanied by declining ability to reproduce, increasing incidence of health problems, and declining longevity in modern dairy cows. Genetic selection for increased milk yield is increasingly viewed as increasing profit at the expense of reducing animal welfare. The welfare problems should be addressed before there is widespread condemnation of breeding and management practices. A sustainable breeding goal aimed at improving fitness and tolerance to metabolic stress is recommended.

**Keywords:** dairy cows, fertility, health, welfare, genetics

### INTRODUCTION

The dairy industry's goal has always been to produce quality milk for the consumer market. In many countries yield per cow has more than doubled in the last 40 years. The dramatic increase in yield per cow is due to rapid progress in genetics and management. The average ECM (Energy corrected milk) yield for Swedish dairy cows increased from 4200 kg to 9000 kg between 1957 and 2003 (1). Data from National Milk Records in the UK show an increase in average yields of dairy cows of about 200 kg/year from 1996 to 2002 and 50% of the progress in milk yield is attributed to genetics (2).

The picture is similar in the US where between 1993 and 2002 the average milk production/cow increased by 1287 kg and 708 kg of this increase, or 55%, was due to genetics. Interesting to note that, until mid-1980s most of the increase in milk yield was the result of improved management, in particular better application of nutritional standards and improved quality of roughage. Since then, genetics became the major factor due to effective use of artificial insemination (AI), intense selection based on progeny testing of bulls and worldwide distribution of semen from bulls with high genetic merit for production.

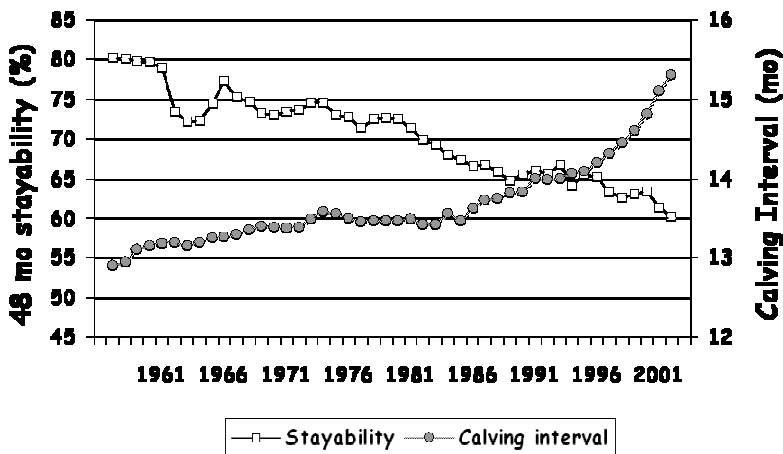
There are several practical reasons why high production should be viewed with concern: a) the increase in milk yield has been accompanied by declining ability to reproduce, increasing incidence of health problems, and declining longevity in modern dairy cows; b) substantial antagonistic genetic correlation exists between milk yield and fertility and between milk yield and several production diseases indicating that, if selection for production continues unchanged, further genetic deterioration in fertility and health is expected; c) high disease incidence, reduced ability to breed, decreased longevity and modification of normal behaviour are indicative of substantial decline in the welfare of dairy cows; d) the success of dairy industry depends upon public perception of its products and production methods and increased public concerns regarding modern animal agriculture, particularly animal welfare, puts sustainability of dairy industry at risk.

## DISCUSSION

### *Declining fertility, health and longevity of modern dairy cows*

Calving interval increased from < 13.0 months to > 14.5 months and number of inseminations per conception from 2.0 to > 3.5 from 1980 to 2000 in 143 US commercial herds (3). A decline in pregnancy rate to first service of 0.5% per year between 1975 and 1997 was reported in the US (4). Poor reproductive performance often leads to premature culling of dairy cows.

The decline in fertility, reflected in increased calving interval, and in longevity, measured by satiability, in Holstein cows in the Northeast US from 1957 to 2002 are shown in Figure 1. Average milk yield per cow over the same period increased from about 5000 kg to 11000 kg at a relatively steady rate of about 150 kg per year. Interesting to note that average calving interval increased by only 0.5 mo from 1960 to late 1980s and by almost 2 mo in the last 15 years and the trend seems to reflect the pattern of rate of genetic gain. Proportion of cows still alive at 48 mo of age (stay ability) decreased from 80% to 60% between 1957 and 2002 indicating a substantial decrease in length of productive life of modern dairy cow.



**Figure 1.** Average calving interval and 48 months stay ability over time for Holstein cows in Northeast US

Declining reproductive efficiency is not limited to the US. In the UK (5) pregnancy rate to first service decreased from 56% in 1975–82 to about 40% in 1995–98, a decrease of about 1% per year. Similar decreases in conception rate and other reproductive measures have been reported in Sweden (6) and many other countries.

The incidence of production-related diseases has increased greatly over the last decades. A case in point is lameness. Its incidence increased in UK dairy herds from lactational incidence rate (LIR) <10% reported prior to 1980 to >20% after 1990 (7). For the US, Guard (8) reported a 38% LIR and estimated direct cost due to lameness in a 100-cow herd to be \$7,600.

Ingvartsen et al. (9) reviewed the literature on the relationship between milk performance and health in dairy cattle. The review dealt with production diseases as defined by Kelton et al. (10): dystocia, parturient paresis, ketosis, displaced abomasum, retained placenta, ovarian cyst, metritis,

mastitis and lameness. The review of 11 epidemiological studies showed clear evidence that cows with high yield in the previous lactation are at increased risk of mastitis and ovarian cysts in subsequent lactation, but for other diseases the phenotypic association was weak due to the large variability between studies. These results led Ingvarsten et al. (9) to conclude that examining the relationship in terms of cause (level of milk production) and effect (disease incidence) is inadequate as cows producing more milk are also likely to eat more and make greater use of their body reserves in early lactation (11).

### ***Unfavourable genetic association of milk yield with fertility and health in modern dairy cows***

Many published studies (2, 5, 6) reported strong unfavourable genetic associations between milk yield and fertility measures – indicating that the decline in fertility observed on the dairy farms is, at least in part, an unwanted consequence of successful selection for higher yields.

Ingvarsten et al. (9) reviewed 14 genetic studies on the relationship between milk performance and health in dairy cattle. These studies showed an unfavourable genetic correlation between milk yield and incidence of ketosis (0.26–0.65), ovarian cyst (0.23–0.42), mastitis (0.15–0.68) and lameness (0.24–0.48) indicating that continued selection for higher milk yield will increase LIR for these production diseases.

### ***Declining adaptability of modern dairy cows***

It is clear that selection for production may lead to problems in health and fertility. As animals tend to adapt to the environment they are selected in, it is likely that selection for increased yield may also lead to environmental sensitivity expressed as genotype  $\times$  environment ( $G \times E$ ) interaction. Castillo et al. (12) and Kearney et al. (13) showed that the magnitude of the antagonistic genetic correlations between milk yield and somatic cell score and between milk yield and conception rate were significantly higher in a poor environment relative to a good environment. Dairy producers in several grazing countries have expressed concern regarding the declining fertility of cows with increased Holstein genes. Harris and Winkelman (14) and Verkerk et al. (15) reported significant differences between cows of New Zealand origin and North American origin for conception rate, services per conception, and days to first service. These changes in the genetic correlations between traits are indicative of genotype by environment interaction; suggest a decline in fitness and adaptability associated with selection for increased production and leads to welfare problems, especially when the animals are exposed to a poorer environment.

### ***Major welfare performance indicators***

One definition of animal welfare put forward by Broom (16) states that “The welfare of an individual is its state as regards its attempts to cope with its environment”. Animal welfare ranges from poor to good and an objective way to assess it is in terms of directly measurable biological functions such as reproductive success, disease incidence, survival and behavioural changes (17). Duration, prevalence and severity are aspects that need to be considered to assess the importance of any welfare indicator. A total of 22 scientists participated anonymously (Delphi method) to develop a conceptual framework for assessment of farm animal welfare, to identify the major welfare performance indicators and rank them in order of importance. For dairy cattle the major welfare indicators in order of priority were: lameness, mastitis, other metabolic disorders, sub-fertility, and longevity (17).

### ***Sustainability of dairy industry in a changing culture***

For the most part of the 20<sup>th</sup> century the goals for animal agriculture were increased production and increase efficiency to satisfy a consumer market that demanded an abundance of animal products at low cost. Under these circumstances, it is not surprising that the main aim of dairy cattle breeding for the last 50 years was to improve production and efficiency, with genetic selection focused on increasing milk yield. This goal has received wide support because, other things being equal, it should optimize the use of resources, increase farm profit and reduce cost for consumers. However, this approach has also led to undesirable and ethically problematic consequences, particularly with respect to welfare and adaptability of modern dairy cow. Today, the attitude toward farm animals in the developed countries has changed and other issues, particularly animal welfare, are of primary public concern. Genetic selection for increased milk yield is increasingly viewed as increasing profit at the expense of reducing animal welfare. The sustainability of dairy industry is directly related to public acceptance of its production practices. Unless remedial measures are taken, there is a real danger for the dairy industry to find itself out of steps with public values, loose market share and experience serious economic hardship.

### ***Selection for high production and metabolic stress***

As the genetic ability to produce milk increases, more cows have sub-fertility or production diseases. As more cows are culled for health or fertility reasons, the productive life of modern cows is rapidly declining. The associations between increasing production and deterioration of the most important indicators of welfare are well documented, but less is known about the biological mechanisms behind these relationships.

One of the first attempts to explain the negative collateral consequences of selection for increased production were presented by Goddard and Beilharz (18) who suggested the “Resource Allocation Theory”. The resources an animal have are limited and as a result, if output is increased through one biological process, such as producing more milk, other functions will be affected such as fertility, maintenance, movement, immune defence, etc. The resources that one process demands can be increased to a certain extent. Due to management, such as increasing access to feed and nutrients, fitness of the animal could increase until resources became limited again. Any further increase in fitness would imply a reallocation of resources and thus modify other outputs such as disease resistance or behaviour (19). Reviewing the negative side effects of selection for high production Rauw et al. (20) concluded that “when a population is genetically driven towards high production,”...” less resources will be left to respond adequately to other demands like coping with (unexpected) stressors; i.e. buffer capacity is [negatively] affected”.

To address the growing perception that the pursuit of ever-increasing milk production is detrimental to cows’ welfare, Ingvarsten et al. (9) developed a framework for future research. The framework links the genotype, nutritional environment and management of the cow through its metabolic status to fertility and disease susceptibility and suggests that mobilization of body reserves has the potential to be the key factor. High producing dairy cows need to mobilize body reserve to support their milk production. In early 1/3 of the lactation period, until energy intake catches up with the requirements, high producing cows enter a state of negative energy balance (NEB) losing excessive amounts of body condition. The terms “metabolic load” (ML) and “metabolic stress” (MS) are used to describe the effects of high production on dairy cows. The ML is defined as “the burden imposed by the synthesis and secretion of milk” and MS as “that amount of ML which cannot be sustained, such that some energetic processes, including those that maintain good fertility and general health, must be down regulated.” The extent and type of

down regulation would be indicative of the degree of MS. The term ML is often used to describe only the part of the total energetic burden of lactation that is met by mobilization of body reserves, i.e., ML is the same as NEB.

Selection for yield increases the demand for energy and also shifts the priority in partitioning of energy to support milk synthesis. It also increases feed intake but, with a genetic correlation between yield and feed intake ranging from 0.46 to 0.65 (11), the gap between energy input and output during early lactation is increasing. There is little evidence for more efficient digestion or utilization of metabolizable energy in high-genetic-merit cows; so, the correlated response to selection for yield is increased body-tissue mobilization and increased ML. Unfavourable genetic correlations were reported between NEB and interval to first luteal activity (21), incidences of digestive (milk fever, ketosis, displaced abomasum, diarrhoea and indigestion) and locomotive (laminitis, leg problems, hock problems, and inflamed thigh) problems (22), and average somatic cell count (23). These reports suggest that a major part of the decline in health and fertility observed over time is the result of the increased MS associated with the success of the genetic selection for milk yield.

Management practices that may also increase the gap between energy input and output are expected to increase the MS and negatively affect fertility-health-welfare complex. Strategies such as increased milking frequency or administration of BST should be carefully evaluated with respect to their impact on the welfare on modern dairy cow before being widely implemented. Another risk of increased MS would occur when cows are not in optimal condition at parturition. This risk may not be high in an intensive dairy system where high concentrate feeding is the norm. However, within European Community there is increased pressure towards more extensive and sustainable dairy production systems that maximizes the use of forages. The protocol for organic dairy farming in Sweden and other Nordic countries includes restrictions concerning concentrate feeding and stipulates a high proportion of dry matter intake from forages. The risk of cows not being able to recover the condition lost in early part of lactation prior to next calving is greater in these systems. This would especially be the case when the cows within such a system were those which had previously been intensively selected for milk production in intensive systems. Restoring adaptability may be required if organic dairy farming is to expand and prosper.

There is even greater concern that, if the single-goal genetic selection for milk production continues unchanged, the future welfare of the dairy cow may be severely compromised. Consideration of animal well-being in any livestock production system is a determining factor for its social acceptability and, therefore, its sustainability (24). The double-edged sword of genetic yield improvement and associated metabolic-stress symptoms raises important challenges for sustainability of the dairy industry.

Welfare assessment is based on establishing norm values for animal related parameters (health, fertility, longevity, behaviour, etc.). With selection, the genetics of the entire dairy population is continuously and cumulatively changing and the genetic improvement for production is accompanied by genetic deterioration of major welfare indicators. Unless this deterioration is stopped, appropriate norm values with long term relevance can never be established.

### *Selection for improved welfare in dairy cattle*

In regard to breeding programs, The United Kingdom's Farm Animal Welfare Council (25) in its report on dairy cow welfare, recommended the following:

*“Achievement of good welfare should be of paramount importance in breeding programs. Breeding companies should devote their efforts primarily to selection for health traits so as to reduce current levels of lameness, mastitis and infertility; selection for higher milk yield should follow only once these health issues have been addressed.”*

Broom (26), pointing out positive correlations between milk production and the major welfare problems in dairy cows (lameness, mastitis, impaired reproduction, inability to show normal behaviour), stated:

*“Genetic selection has not taken adequate account of the adaptability and welfare of cows. Current trends towards ever greater milk production should not be continued unless it can be insured that welfare is good. It is important to the dairy industry that welfare problems should be addressed before there is widespread condemnation of breeding and management practices.”*

The major advantages of genetic improvement for any trait are that changes are cumulative, permanent, cost-effective and sustainable. This is true for the selection trait as well as for correlated responses on other traits. As pointed out, these very advantages have facilitated a rapid increase in milk yield per cow and detrimental effects on the welfare of the animals when breeding objectives have centred on production, with little attention given to fitness traits, such as fertility and health.

## PRACTICAL IMPLICATIONS

The unfavourable genetic relationship between milk production and welfare indicators means that the most effective route to stop the decline or even improve welfare is by developing and adopting a selection index in which welfare related traits are included and appropriately weighted. With such an index the genetic progress for any of the traits considered is smaller than if selection is for a single trait, but overall economic response is greater than in single trait selection.

Animal welfare is often portrayed as opposed to animal production (27) and selecting for welfare traits is assumed to be uneconomical. This is not the case. The current breeding goal in the UK includes milk, fat and protein yields plus lifespan. These traits are combined into Profitable Lifetime Index, or £PLI. Calculations suggest that expansion of £PLI to include mastitis resistance and measures of fertility (calving interval) could increase economic response to selection by up to 80%, compared with selection for milk production alone (28). Selection on such an index could also halt the decline in fertility and mastitis resistance, compared with selection for milk production alone. This example illustrates that it should indeed be possible, through genetic selection to address the welfare without a reduction in profitability.

One example of a successful multitrait selection comes from Sweden and other Nordic countries where breeding goals have been formulated to include not only production but also fertility and health for the last 20 years. By implementing a more balanced selection goals it has been possible to limit the decline of fertility in SLB breed to about half of what has been observed in other Holstein populations and prevent it in SRB breed which is much less influenced by germplasm from outside Scandinavia. Resistance to mastitis follows similar trends.

Short term, all breeding organization should use available records to include fertility, health and longevity in the breeding goals. The selection method should be via a selection index in which greater emphasis should be placed on all fitness related traits relative to production traits.

Management strategies to reduce the negative effect of metabolic stress should be developed and implemented. For example, reduced fertility resulting from long anoestrous postpartum or low

conception rate may be just consequences of cows coping with MS. Hormonal treatments used more and more, particularly in North America, to kick-start the reproductive cycle or to solve fertility problems may not be good solutions as they may further compromise cows' welfare by preventing them from using one of the few coping mechanisms left. Extended lactation is increasingly used to manage high yielding cows and it may represent a better strategy to manage low fertility in modern cows without additional welfare cost.

In order to improve the welfare and adaptability of dairy cows through genetic selection long term, the cooperation of breeding experts, geneticists, epidemiologists, nutritionists, ethologists and others concerned with animal welfare problems is required. Sustainable breeding goals aimed at improving fitness and tolerance to metabolic stress is necessary to prevent the decrease in the quality of life of the animals and, perhaps, enhance it. The effectiveness of a selection program to improve welfare should be enhanced if selection acts directly on causes of poor welfare and not only on its symptoms. To implement such a program, research is needed to clarify the relationship between production, NEB, MS and welfare indicators and to develop practical methods for measuring NEB and MS. This research should identify traits directly related to welfare status, such as NEB, body condition score, onset of cyclicity after calving, etc., and, ultimately, provide better selection tools to improve welfare status in dairy cows.

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## NEW CHALLENGES FOR ENVIRONMENTAL PROTECTION IN TERMS OF INTENSIVE ANIMAL PRODUCTION

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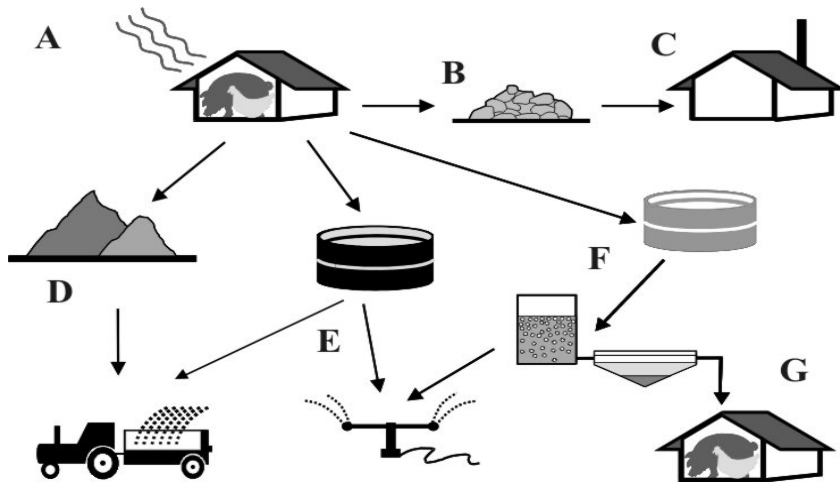
### SUMMARY

Modern livestock require the proper handling of the large volumes of manure produced. The main concerns are nutrients which are often in excess of the capacity of the local environment resulting in air and water pollution. Treatment can play a key role in the removal of these surpluses either by breakdown or by exporting as useful products – a wide range of technologies is already well established. However it is the hygienic aspect of manure handling that may pose the greater challenge as concerns grow over the spread of disease and the potential contamination of water and food crops. Thermal treatment represents an effective and versatile option but it is often overlooked due to the anticipated expense. Heat recovery may yet make this approach accessible to the farming systems especially if the process is coupled to an anaerobic digester which can potentially produce an energy neutral process.

**Keywords:** manure management, thermal treatment, anaerobic digestion, food safety, disease transmission, pollution

### INTRODUCTION

Food safety and environmental quality are foremost topics in the public's mind. The two are intimately linked because food production cannot be separated from the land, itself an integral part of the natural environment. In the late twentieth century, farmers were encouraged to intensify in order to remain profitable. However, concerns on hygiene issues have been raised by a series of food scares from the microbiological contamination of agricultural food products (salmonella, e-coli, campylobacter and also BSE). In addition there have been notable outbreaks of diseases affecting the animals themselves including foot and mouth, classical swine fever and more recently, avian influenza. Policies that aim to encourage efficient production of inexpensive food may threaten animal health, food safety and the natural environment. However, ill-considered legislation to reduce pollution and/or promote food safety can significantly damage farming activity whilst also failing to achieve the intended purpose. Furthermore, moves to protect food quality by restricting the use of livestock manures on crops can undermine measures for environmental protection by limiting the opportunity to usefully recycle and thus aggravating the problem of nutrient excess.



**Figure 1.** The principal wastes streams from livestock farming: A – emissions, B – fallen stock, C – wastes related to the rendering process, D – solid farm wastes including bedding, E – liquid manures, F – other wastewaters and G – water reuse

The wastes produced from livestock farming are summarised in Figure 1. In terms of volume, fallen stock is a very small percentage but it is often the cause of particular concerns over hygiene. The largest volumes are often of liquid manures that present the main problems to the environment due to the mobility of the nutrients contained. Solid wastes (including farmyard manures or FYM) are considered to be more stable but can present problems in handling. On some farms, there is the separate collection of a more dilute wastewater (also known as dirty water). This can be potentially treated to enable use around the farm for a limited number of duties – an important consideration where water is a limited resource and recycling is encouraged. There is no shortage of research effort or of available techniques to deal with the manure management which lies at the heart of the problem (Burton and Turner, 2003; Burton 1996 and 2006). Rather it is an issue of *strategy*: trying to meet multiple targets in the absence of clear procedures to evaluate and compare the methods available along with their unwanted consequences.

## THE ENVIRONMENTAL IMPACT FROM LIVESTOCK FARMING

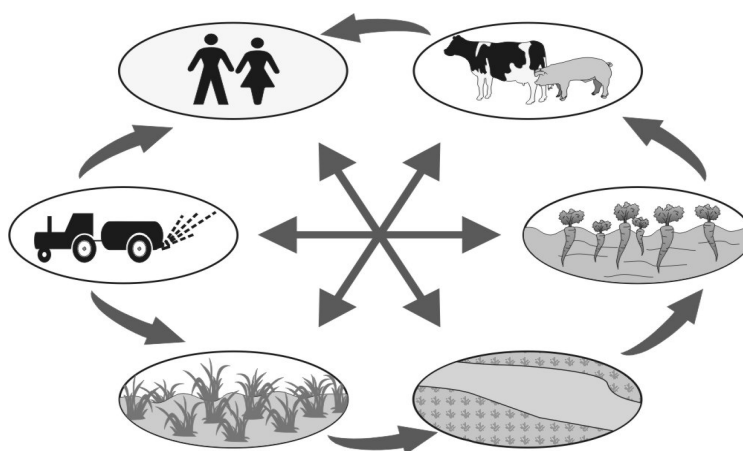
There are many papers that cover in detail the various environmental effects from raising animals (e.g.: Burton, et al, 2000). Such impacts are from the wastes produced along with the related emissions and odour nuisance.

- The impact on air quality: effect of the management technique on emissions including ammonia, methane, nitrous oxide and particulate matter.

- Impact on water quality (surface and underground): effect of management technique on release of nutrients (nitrogen, phosphorous and organic materials). Risk of water contamination by pathogens.
- Impact on soil quality: changes in soil structure as a result of implementing the proposed measures both short and long term. Expected accumulation effects including phosphorous and heavy metals.
- Effect on nuisance factors including offensive odours and impact on local community.
- Contribution to animal and farm hygiene. Effect on the farm bio-security and on the spread or abatement of infectious animal diseases. Risks to farm staff and local people.
- Impact on the quality of food produced. Contamination risks of crops from zoonotic pathogens – both by direct applications of manures and by indirect routes (via air, soil or water).

These can be more simply grouped as nuisance, pollution or hygienic concerns. In the case of odour nuisance, this implies both revised management practices such as timing or method of land spreading, or the introduction of an effective treatment regime such as aeration (Burton et al, 1998). Although odour has only a small effect on the environment, it is often the principal factor behind local complaints of livestock farming.

The greater concern in terms of the environment is from water, air and soil pollution. This includes emissions of ammonia, methane and nitrous oxide to the air, the release of ammonia, phosphorous and organic matter to surface waters and contamination of underground water by nitrates. Many of these unwanted effects are often the result of the poor use of nutrients – sometimes good housekeeping measures alone such as improved collection and storage and/or manure management plans can greatly help. In cases where there are local or regional excesses of manure nutrients such as phosphorous or nitrogen, some form of treatment is required with the removal of surpluses as a concentrate or (in the case of nitrogen) as the products of the nitrification / de-nitrification process (Pahl et al, 2003).



**Figure 2.** There are many transfer routes for pathogens moving through the environment. Some of these may be minor yet still cause considerable public concern.

In recent years, hygienic issues resulting from the pathogens invariably present in animal wastes have been the cause of increasing concern. The resulting impacts include health risks to the animals and people either directly or indirectly via water or food produced (Figure 2). The extent of such risks is the source of much research and discussion but it may be that public fears of ill health are greater than the actual risk presented. Hygienic impacts can be grouped under four headings:

*A: direct risk to farm staff and people nearby.* The perceived risk in this case may be much higher than the actual risk and there have been few verified cases of illness as a result of this route. Farm staff in regular close proximity with animals are at highest risk but this can also be the result of the internal environment of the animal buildings (e.g.: dust inhalation, high ammonia concentrations etc) as much as from a direct infection from the waste materials present. However, the recent cases of avian flu generating illness in local people emphasises the problem and bio-security at least will need to be reviewed. Outside the farm, the main impact on local people will be via the land spreading practice and the aerosols sometimes produced as a result. The fact that this can cause offensive odours is not disputed but this should not be confused with an exposure to an infective dose of a zoonotic disease. Nonetheless, public pressure evoked from odour concerns can quickly turn to health matters and scientific argument alone may not be enough to resolve such disputes.

*B: contamination of food crops.* Again the perceived risk may be higher than that in reality. Furthermore, the avoidance of land spreading manures on certain vegetable crops will be no guarantee that they will not be contaminated from other environmental sources such as wild animals or birds. One might add that there may be equal or greater concerns over the practice of irrigating river water onto food crops. Clearly though, the application of manures to certain vulnerable crops such as lettuce can increase the risk of contamination from a range of common pathogens such as salmonella, e-coli or campylobacter. The issue is that, despite the organic credentials, there may be increasing reluctance to apply untreated manures to a much wider range of vegetable crops including those for which there is little evidence of contamination. Land spreading potentially represents the most environmentally-friendly method of manure disposal so long as suitable cropland is available for the purpose; the areas available may be greatly reduced if fears of food contamination from pathogens are not calmed.

*C: contamination of water supplies.* There have been cases of drinking water becoming contaminated by effluents from agriculture with the resulting illness affecting local people including fatalities (e.g.: Walkerton, Canada: Guan and Holley, 2003). Such incidents are relatively few and often the result of accidental discharge. However, it is all too apparent that manures can enter surface waters adding a bacterial load to the system – e.g.: from run-off especially if heavy rainfall follows land applications. Various methods can be proposed for better management of land spreading practice to protect rivers and streams but in some high risk areas this may not be considered enough and the farmer may find himself with greatly restricted options. If water quality is likely to be effected, the reaction of local people and politicians can be expected to be very determined and restrictive measures may well follow.

*D: disease spread amongst farm animals.* The spread of zoonotic diseases to people will always cause the greatest reaction, but often, it is the risk to animals that is the greatest. Within the farm, the transfer of disease via manure is both easily understood but there is an additional dimension in the case of grazing animals and land spreading on grassland. In this case, there is a real transmission risk to neighbouring farms. Fortunately, many of the main notifiable animal diseases of concern are rarely present. Nonetheless, in cases of disease outbreak amongst farm animals and in the absence of good bio-security and good waste management there is a much

increased rate of spread. Furthermore, following the outbreak of a notifiable disease at a livestock farm, there is the additional problem of safely disposing of any waste material collected or already in store which must be deemed contaminated.

### THE ROLE OF TREATMENT PROCESSES IN COMBATING HYGIENE RISKS

In response to disease related risks, one can consider either storage, drying or treatment of the various wastes to reduce or eliminate the pathogenic agents. Storage represents the simplest option but care must be taken to prevent re-contamination from further addition of raw wastes. The typical farm store can fulfil this role once full and left for a period of time. However, the length of time can be considerable especially during cold weather; for example, 20 days storage was found to be enough time to inactivate *Cryptosporidium* oocysts in cattle slurry at 20 °C but this increased to 90 days at 4°C (Svoboda et al, 1997). Some bacteria, viruses and other pathogenic agents have shorter survival times in dry conditions and/or when exposed to oxygen although the process of drying itself may be a factor. Survival in solid manure has indeed been reported as less than for liquid manure and once land spread (especially during warmer weather), but other results contradict this trend possible the result of conditions allowing the formation of spores (Burton and Turner, 2003). The net effect of such uncertainty is to specify storage times running into months when there is a particular disease risk present.

Various studies have concluded that the treatment options come down to either chemical use (such as disinfectants) or heat application (Turner and Burton, 1997). In some cases, the exposure to pathogens to biological treatments (especially aerobic systems or at temperatures above 50°C) also results with a substantial reduction in numbers. The main limitation with such processes lies with the concept of back-mixing which is almost invariably present – as such; there is almost inevitably the re-inoculation of treated wastes by the raw material entering the system which partly negates the benefit of treatment. The problem is largely avoided in batch processes but at the price of a system that is very difficult to control and unsuitable for large volumes; sequential batch reactors (SBR's) represent a useful compromise. Biological treatments (both aerobic and anaerobic) will remain an important option because of their effectiveness in removing reactive organic matter and (in the case of nitrification and de-nitrification) the removal of nitrogen as well (Beline et al, 2004). Furthermore, the related settling options enable the removal of phosphorous.

The use of chemical disinfectant on farms is commonplace but unpopular because of (a) the implied costs (b) the hazards of handling and (c) the detrimental environmental impact. It is noted that chemicals are less effective within solid material where penetration is inhibited – bacteria within such material might survive such treatment. For manures where there is almost always the presence of ammonia, a bactericidal effect from the chemical can be expected in addition to that of heat, oxygen or biological activity whichever is the principle agent of the process (Turner et al. 1999).

Raising the temperature is almost universally effective in accelerating the reduction of pathogenic agents; the higher the temperature, the faster and further the decline in numbers. Above 50°C, the time required is down to hours and above 70°C, minutes may be enough to ensure total removal of a pathogenic agent. The attractiveness of thermal treatments lies with its inherent effectiveness – given time, all the treated matter can be brought to an inactivation temperature where the microbes and spores contained are destroyed. Modelling of heat transfer systems is generally easier and more reliable than of chemical (mass) transfer and both much easier than of biological systems. In addition, depending on the method of heat application, a

rapid temperature change is possible adding a thermal shock factor that further enhances the effectiveness of the process.

The clear disadvantage to thermal processes lies with cost and the related implication of an environmental penalty in the energy consumed. This may be addressed in two ways: firstly by the use of heat recovery technology and secondly by utilising the heat already available within the livestock waste. In some cases, a cost neutral process (in energy terms) may be possible which would be a crucial pre-condition before such technology could be considered for general use within the farming system.

### THE AVAILABILITY OF ENERGY FROM LIVESTOCK WASTES

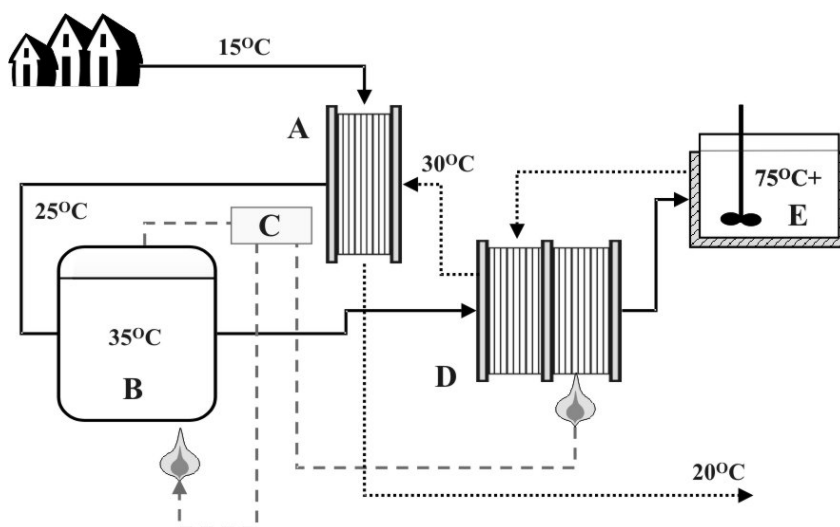
Aerobic biological treatment processes for livestock manure are exothermic and are accompanied by a rise in temperature. However, in many cases, the availability of this “free” heat is not translated into any real benefit although general warming can be expected to enhance microbiological activity. Some composting systems do reach 70°C to enable a final pasteurisation of the product but others struggle to reliably reach 50°C. For liquid manure systems elevated temperatures are not easily achieved and the general preference is to operate at mesophilic temperatures (20 to 40°C). Some anaerobic digesters have been run at 55°C with the benefit of enhanced pathogen reduction and possibly also increased degradation, but sustaining the elevated temperatures can consume a high proportion of the biogas in cold regions.

The potential heat from the aerobic degradation of pig manure is around 14.5 MJ per kg of oxygen *consumed* (Evans et al, 1982). This reflects similar values measured for a range of organic compounds – 16.7 for methane, 12.8 for n-octane and 13.0 for benzene (Perry, 1973). Typical organic matter in manure is hard to define but assuming a ratio of 2:1 H:C, one kilogram of pure organic matter fully oxidized will typically need 3.4 kg of oxygen. Various studies (e.g.: Williams et al, 1989) indicate that one kilo of the dry matter in pig slurry will contain reactive organic matter needing around 400g of oxygen for complete oxidation in five days (i.e., its BOD<sub>5</sub> or biological oxygen demand content) – this implies a readily available heat output of 5.8 MJ per kg of dry solids fed to the reactor. In reality, the presence of other oxidizable components in manure, especially nitrogen, will enable a slightly higher value.

In the case of anaerobic digestion, there is little heat of reaction and sometimes, heating must be provided to sustain an adequate reactor temperature. The equivalent energy in this case is released as biogas. Keeping with pig slurry, this can yield 340 to 550 litres of biogas per kg of volatile solids (VS) fully digested (Burton and Turner, 2003). Taking a median figure of 450 and a biogas containing 60% methane and pig solids containing 70% VS, the maximum methane yield is around 190 litres per kg of total dry solids fed to the digester. In combustion, one volume of methane will require one and a half volumes of oxygen or 285 litres which is equivalent to 342g if we take the gas density of 1.2g/litre. Applying the previously cited figure of 16.7 MJ per kgO<sub>2</sub> consumed for methane gives the energy from the methane produced from the one kilogram of manure solids as 5.7 MJ. This is not surprisingly similar to the figure of 5.8 MJ of heat released per kg of dry piggy solids fed to an aerobic reactor. The implication is that the anaerobic process is barely exothermic with around 95%+ of the potential thermal energy available going into the methane produced – this is indeed what is generally observed. However, the biogas that can be produced is a usable source of fuel equal to 290 MJ per tonne of typical piggy slurry with a dry matter concentration of 50 kg per tonne.

### MANURE PASTEURISATION AND STERILISATION SCHEMES

The application of heat alone in a thermal process has been demonstrated as an effective treatment against a range of pathogens. Tuner et al (1999) demonstrated a reduction by four log<sub>10</sub> units of a range of viruses exposed to temperatures between 55 and 65°C for a nominal 5 minutes. The same research demonstrated the option of heat recovery in excess of 80% by the use of heat exchangers in which the treated effluent warmed that entering the system. Taking the specific heat capacity as 4000 kJ per deg.C per tonne, the heat required to warm an aqueous effluent from an ambient of 15 to 65°C is around 200 MJ per tonne (or m<sup>3</sup> on the basis that the liquid density is close to unity). Energy costs if available at 10 cents (euro) per kWh would be a prohibitive 5.60 euros per tonne treated. If the effluent in question was 5% piggery slurry, then there would potentially be enough energy via the potential biogas production to provide this requirement allowing for some losses and inefficiencies. However, if there is 80% heat recovery, then the energy demand falls to 40 MJ per tonne reducing the energy cost to around one euro per tonne. Moreover, this could easily be covered by the potential biogas produced. The broad scheme is set out in Figure 3:



**Figure 3.** Schematic of a thermal treatment plant for effluents produced from a livestock farm. Heat exchanger (A) is for the optional pre-heating of effluent prior to anaerobic digester, B. The biogas (C) both sustains the digester and the principal thermal process (E). Heat recovery is via the second heat exchanger, D.

The scheme is based around anaerobic digestion with no aerobic stage and thus no removal of nitrogen; such a scheme may be of greater interest for farms wishing to utilise the full agronomic value of the manure but with a reduced risk of crop contamination. Here there is the additional consideration of maintaining the temperature of the digester. On the basis that the final treatment temperature will be over 70°C, it's quite possible that once the process is established, heat recovery will reduce the demand for gas for thermal treatment and some will be left over for external uses leaving the system as a net gas generator. There are several alternatives including a combined approach with an anaerobic digestion unit followed by an aerobic treatment system.



The drawbacks of these schemes lie principally with the cost and running of the equipment, especially the heat exchangers. These exist in many designs but all are prone to blockage and fouling over the course of time. Pre-screening (and even centrifugation) of the raw effluent may be required with the removed solids being separately added to the digestion processes. Fouling will progressively reduce heat transfer performance, and periodic cleaning will be required to remove deposits.

## CONCLUSIONS

- The principal environmental concerns from livestock farming are related to the management of the wastes produced. The main negative impacts are the pollution of air, water and soil, health and hygiene issues and odour nuisance.
- A wide range of management techniques already exist that enable the safe handling and disposal of livestock wastes. Observing good practice in the collection and targeted application of manures represents a first step for all farmers. Where there are surpluses of nutrient over local crop needs, treatment may be required to enable the removal of the excess. This includes aerobic treatment, anaerobic digestion, composting and separation processes with the export of manure products.
- The patchy uptake of manure treatment across Europe demonstrates some reluctance to use this option which is often due to cost. Difficulties in meeting legislation and local pressures are the main reasons why some farmers have been adopting such technologies. However there are also some benefits that offset the costs including biogas production, the reduced need for chemical fertilisers and the option to sell manure products as organic fertilisers.
- It is concerns over hygiene that ultimately may determine the strategy followed for manure management. From the farming point of view, such fears encompass the spread of notifiable diseases amongst livestock. The greater pressure may come from the risk of water or crop contamination. This last factor may create new problems in the safe disposal of manures onto farmland; the use of treatment may again become an important step in the process.
- Decontamination of manure can be achieved by storage, drying, chemicals, raised temperature or biological systems. Thermal treatment of effluents represents the most reliable system especially if there are particular disease risks. The implied costs can be reduced by heat recovery and if coupled with anaerobic digestion, an energy neutral process is theoretically possible.

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## ENVIRONMENTAL STRESS AND REPRODUCTION IN COWS AND SHEEP

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### SUMMARY

The husbandry of ruminants has not kept pace with genetic advances in potential performance. Adequate feeding and housing have great influence on animal comfort and reproductive performance. Increasing severity of mastitis or lameness both reduce fertility, in spite of treatment. In cows and sheep, reductions in LH pulse and/or surge patterns contribute to decreased fertility. For a short-term solution, animals need to be fed better, provided with improved housing, and subject to fewer production-related diseases. In the long-term, genetic strategies such as by increasing persistency of lactation in dairy animals and by increased use of gene markers should provide a solution.

**Keywords:** genetics, feeding, housing, LH, production diseases, persistent lactation

### INTRODUCTION

The aim of this paper is to high-light the pressure points (vulnerable links) in the interaction between the environment and reproductive performance in cows and sheep. This will be achieved by reflecting on

- the problems encountered;
- the principal mechanism by which these are mediated;
- suggesting appropriate solutions.

‘Progress’ from extensive to intensive husbandry within the cattle and sheep industry has led to animal under-performance (and heightened public concern in some countries). For example within the dairy cow population, there is substantial evidence to show that production-related diseases reduce both fertility and potential milk yields (Borsberry & Dobson 1989; Bareille et al 2003).

### THE PROBLEMS

#### *Genetics and breeding*

Approximately 60–65% of the increased yield per dairy cow over the past 20 years has been achieved by selective breeding i.e., via genotype. In other words, since 1983 an extra 120–135 kg fat per year is now being produced per cow, 70–89 kg of which are attributable to genetic gain (JP Chenais and F Miglior, *personal communication*). Regrettably this means that the way we look after animals, i.e., ‘husbandry’, is not keeping pace with genetics. Many countries now have a

national dairy herd that produces at least 7000 litres milk per cow in 305-day lactations but concerns are being expressed about a concurrent reduction in fertility (Royal et al 2000; Butler 2003). Our inability to appropriately feed and house these very high-yielding animals is leading to a failure to meet genetic potential, and we must provide realistic solutions soon if we want a sustainable agricultural industry.

There is little information widely available comparing genotype to phenotype success in the sheep and beef industries. Increased prolificacy is one approach to increase sheep productivity; however, considerable research has revealed that incorporation of 'prolificacy' genes in sheep leads to greater embryo losses. For example, within the highly prolific Cambridge breed, pure bred ewes with no copies of the prolificacy gene(s) have 2–3 ovulations and subsequently 2–3 lambs; ewes with one copy have 4–5 ovulations and 4–5 lambs; whereas, those with 9–13 ovulations produce only 1–2 lambs (DAR Davies, *personal communication*). Furthermore, there are some breeds of sheep, for example the Inverdale, that possess a gene motif that can result in very poor development of the uterine tract, obviously resulting in infertility of part of the population (Davis et al, 1992).

### ***Feeding***

A major characteristic that genetics has introduced into many national dairy cattle herds is the sudden large increase in milk yield in early lactation. As suggested above, one of the most common difficulties encountered by modern dairy cows is achieving sufficient food dry matter intake during the early-mid postpartum period. This is revealed by poor body condition scores in early lactation and a marked improvement in pregnancy rates once dry matter intake increases resulting in positive energy balance (Lucy 2001).

The phenomenon of feed 'flushing' has been incorporated into both sheep and beef husbandry for many years to hasten seasonal breeding periods or overcome oestrus suppression due to suckling (Clark 1934). There are several recipes for increasing fertility by increasing energy availability as day-length begins to decrease in sheep (with or without pre-exposure to teaser rams; Walkden-Brown et al 1999); and weaning, even if temporary, has positive effects on return to optimal fertility in beef cows (Stagg et al, 1998). However, the converse of all these situations is also true, leading to considerable restriction on efficiency in some commercial herds and flocks.

### ***Housing***

Adequate cubicle size and number are important for cow comfort, but while the provision of straw-yards increases dairy cow lying time, a compromise has to be made with an increased incidence of udder infections (Whitaker et al, 2000). Dairy cows housed on (slatted) slippery floors express oestrus less intensively with major impact on pregnancy rates to artificial insemination (AI; Britt et al, 1986). High environmental temperatures (>30°C, either within housing or outside without shade) reduce fertility, and this is even more dramatic when dairy cows are under the pressure of producing high milk yields (Al-Katanani et al, 1999). Appropriate levels and locations of lighting are also important as increasing exposure to light in the cubicle area during winter increases lying time, and would thus hinder signs of oestrus (Phillips et al, 1998).

It is unusual to house sheep or suckler cows in the traditional extensive husbandry practice, although it is accepted that this does occur in some parts of the world. However, there are few documented data in the scientific literature concerning the influence of housing on fertility in sheep flocks and beef herds.

### *Production-related diseases*

Ask any dairy farmer what are the main three concerns regarding the herd's performance and a reply will given: mastitis, lameness and fertility. While there is variation between farms, this is only with regard to the priority of these three aspects. Increasing severity of mastitis (or somatic cell counts, SCC) or lameness both reduce fertility and expression of oestrus in spite of treatment (Dobson et al 2001; Walker et al 2005). Several of these post-partum 'production' diseases have their origins in inappropriate feeding either in late pregnancy (dystocia, milk fever) or early post-partum (ketosis) which may also lead to compromised immunity (mastitis, endometritis). Lameness often arises due to changes of diet before and after calving (resulting in changes in hoof growth), but stone-free walkways, comfortable cubicles (to increase lying times) and absence of slurry-pools (predisposing to digital dermatitis) are all important aspects related to the environment in which cows are kept.

There have been few studies in the UK attempting to evaluate clinical reasons for lowered fertility in commercial sheep flocks. However, one report did high-light an incidence of >15% anatomical abnormalities capable of interfering with establishment of a pregnancy (e.g., uterine tract adhesions, blocked fallopian tubes, mucometra; Winter & Dobson 1992). These abnormalities are probably the result of damage and/or infection incurred during lambing.

### **MECHANISM BEHIND THE PROBLEMS**

Females must produce a fertile egg and attract the male (or an AI technician) at the right time to achieve successful fertilisation. In order to do this effectively, follicles must grow in the ovaries, egg(s) need to be released into the female reproductive tract, and hormones produced not only to control pheromone release but also to prepare the uterus to receive the conceptus and maintain the corpus luteum during the 'maternal recognition of pregnancy'.

In more detail for both cows and sheep, neurotransmitters in the higher brain control gonadotrophin releasing hormone (GnRH) and hence luteinising hormone (LH) pulse secretion. Initially, LH is secreted in small discrete pulses, the frequency and amplitude of which drive growth of ovarian follicle(s) and oestradiol production with consequent pheromone release. Towards the end of the follicular phase, there is a precisely-timed surge of LH secretion. This causes resumption of meiosis in the egg(s), further egg cytoplasmic maturation, ovulation and formation of a corpus luteum in the residual follicular tissue. Sequential exposure to correct concentrations of oestradiol (from the growing follicle) and progesterone (from the subsequent corpus luteum) prepare the uterine environment, and in concert with signals from the conceptus, a successful pregnancy will be established.

In evolutionary terms, establishing a pregnancy is a very high-risk strategy for female mammals to pass genes on to the next generation. It is hypothesised that if conditions are not appropriate, LH pulse frequencies and amplitudes will be disrupted, as well as the timing and amplitude of the LH surge, resulting in failure to initiate a pregnancy. Of necessity, this disruption is usually temporary so that when prevailing conditions improve, normal fertility will resume.

Many examples exist in the literature to support this hypothesis and link in with aspects of the environment referred to above as 'the problem'. In all these situations, there is evidence available in cows and sheep to show that either LH pulse or surge patterns (or both) are disrupted and no doubt contributory to changes in fertility (feeding: Butler 2003; male effect: Walkden-Brown et al 1999; day-length: Karsch et al, 1993; weaning: Staggs et al 1998; heat: Badinga et al, 1994;

mastitis: Schrick et al, 2005, Kaneko and Dobson *in preparation*; lameness: Morris and Dobson *in preparation*). In summary, most aspects of environment-induced changes in fertility are orchestrated by LH secretion profiles which in turn are dependant on GnRH patterns and ultimately hypothalamic neurotransmitter system(s) in the brain.

## THE SOLUTIONS

Pay greater attention to the environment in which we keep animals!

In the short-term, get more food into post-partum animals, provide better housing/environment, and reduce the incidence of production-related diseases. But this is easier said than done; the vast majority of farmers are not wilfully starving and mistreating their animals – with the increasing reduction in fertility this would be an ultimately self-destruction strategy. However, there is evidence to show that some farmers can keep digestive diseases, mastitis and lameness to a minimum: 8% clinical mastitis rate, and 2.5% clinical lameness (Whitaker et al, 2004).

In the longer-term, upon reflection, it is apparent that the genetic make-up of the breeds being farmed has out-stripped any advances in husbandry of cows. Perversely, perhaps we should be using genetic strategies to provide a solution? For dairy cows, this would mean exploiting the heritability for persistent lactation estimated at 0.09–0.18 (Haile-Mariam et al 2003). Increases in persistency will decrease peak milk yield 60–80 days after calving (thus relieving the importance of high dry matter intake in early lactation). It will also allow extension of the calving-calving interval thus reducing the frequency of calving that is the major risk factor for the production diseases that occur within the first 60–80 days postpartum. More thoughtful selective breeding within the sheep industry might reduce the emphasis on major leaps in prolificacy and concentrate more on the survivability of the embryo, fetus and neonate. In both species, increased use of SNPs (genetic markers) have the potential to help.

The problems we currently have are important for mankind in terms of reduced (re)productive performance; not for the animals. They are only responding to the abuse imposed by the environment in which they have been ‘domesticated’. The solution to maintain a sustainable fertile population is now an urgent remit for agriculturalists, veterinarians, geneticists and scientists.

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## IMPACT OF HUMAN-ANIMAL INTERACTIONS ON HEALTH AND PRODUCTIVITY OF FARM ANIMALS

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### SUMMARY

While technical skills and knowledge are important attributes of the work performance of stockpeople, two other important but less well recognised characteristics of stockpeople are their attitude and behaviour towards farm animals. Research has shown that stockperson attitude can affect animal productivity, health and welfare by influencing stockperson behaviour and in turn animal fear and stress. While fear thresholds have been reduced by domestication, fear responses to humans have not been eliminated in farm animals. There is a strong case for utilizing stockperson training courses that target stockperson attitudes and behaviour.

**Keywords:** human-animal relationships, productivity, health, animal welfare, fear, stress, attitudes, behaviour

### INTRODUCTION

Modern farm animals have undergone thousands of years of domestication: for horses (*Equus feus*), asses (*Equus africanus*), camels (*Camelus* spp.), water buffalo (*Bubalus bubalis*) and chickens (*Gallus gallus*) about 5,000 to 7,000 years ago in different parts of Asia and North Africa; for llamas and alpacas (*Lama* spp.), guinea pigs (*Cavia* spp.) and turkeys (*Meleagris gallopavo*) also about 5,000 to 7,000 years ago in various locations in the New World; for domesticated cattle (*Bos primigenius*) and pigs (*Sus scrofa*) about 8,000 to 9,000 years ago at various sites in Asia; and for wild sheep (*Ovis orientalis*) and goats (*Capra agagrus*) about 11,000 years ago in the Near East (Serpell, 1986). Many authors, including Serpell (1986), have proposed that it was unlikely that Paleolithic and Neolithic people consciously domesticated animals for specific economic or practical purposes. Rather animal domestication, at least in the early stages, was probably an unconscious process on the part of humans, in which tame or semi-tame wild animals were gradually brought under increasing levels of human control.

Animal domestication can be viewed as a process by which captive animals adapt to humans and the environment that they provide for the animals (Price, 2002). Since domestication implies change, it is expected that the phenotype of the domesticated animal will differ from the phenotype of its wild counterparts. Adaptation to the captive environment is achieved through genetic changes (e.g. artificial selection, natural selection and relaxed selection) occurring over generations, and environmental stimulation and experiences during an animal's lifetime. Thus domestication can be viewed as both an evolutionary process and a developmental phenomenon (Price, 2002).



Despite thousands of years of domestication, studies of feral and free-ranging livestock have shown that the behaviour of our agricultural animals still closely resembles that of their wild ancestors (see Rushen *et al.*, 1999). Although there are many reported differences between wild and domestic stocks, there is little evidence that domestication has resulted in the loss of behaviours from the species repertoire or that the basic structure of the motor patterns for such behaviours has been changed (see Price, 2002). In nearly all cases, behavioural differences between wild and domestic stocks are quantitative in character and best explained by differences in response thresholds. These comparisons are difficult because of problems in both determining an appropriate wild population and interpreting differences between wild and domestic populations under one environment, in nature or in captivity. However, studies of farmed and wild Atlantic salmon (*Salmo salar*) for example, both reared in either captive or wild environments, indicate that farmed salmon show less predator responses, while also showing increased growth, increased disease resistance and decreased stress responses (see Price, 2002).

Nevertheless, while behavioural differences between wild and domestic stocks are mainly quantitative in character, predominantly explained by differences in response thresholds to stimuli, there is still surprising variation within our farm animal species in their behavioural response to humans (see Hemsworth and Coleman, 1998; Waiblinger *et al.*, 2006). Many laboratory studies have shown that handling can markedly affect fear responses of farm animals to humans (see Hemsworth and Coleman, 1998; Rushen *et al.*, 1999; Waiblinger *et al.*, 2006); however the existence of substantial variation in animal fear in commercial farms illustrates the implications of this aversive emotional state in farm animals on animal behaviour, productivity, health and welfare. While this variation highlights the problem in the livestock industries, it also indicates that there are opportunities to reduce the fear response of livestock to humans. This is the topic of the present review.

In this paper, I will review the current literature on the effects of handling and fear of humans on the stress physiology, productivity, health, and welfare of farm animals. I will also briefly examine the opportunities to reduce fear of humans in farm animals. There are a number of valuable reviews in the literature, such as those by Hemsworth and Coleman (1998), Rushen *et al.* (1999) and Waiblinger *et al.* (2006), which I utilise in this review.

### **LABORATORY STUDIES ON THE EFFECTS OF HANDLING ON ANIMAL FEAR, STRESS, PHYSIOLOGY AND HEALTH**

Negative or aversive handling of pigs, imposed briefly but regularly, not only results in high levels fear of humans, but may also markedly reduce growth and reproductive performance in pigs (Barnett *et al.*, 1983; Gonyou *et al.*, 1986; Hemsworth *et al.*, 1981a, 1986a, 1987, 1996a; Hemsworth and Barnett, 1991). The mechanism responsible for the adverse effects of high fear on the productivity of pigs appears to be a chronic stress response, because handling treatments which resulted in high fear levels also produced either a sustained elevation in the basal free cortisol concentrations or an enlargement of the adrenal glands, together with depressions in growth and reproductive performance. It is well known that the long-term activation of the hypothalamic-pituitary adrenal axis can have marked effects on efficiency of growth due to the catabolic effects of ACTH and corticosteroids (Elsasser *et al.*, 2000). Corticosteroids also support the synthesis and action of adrenalin in stimulating glycogenolysis and lipolysis (Matteri *et al.*, 2000). Stress-induced changes in the secretion of pituitary hormones have been implicated in failed reproduction (Clarke *et al.*, 1992; Moberg, 2000). Seabrook and Bartle (1992) also reported depressions in the growth of

pigs following aversive handling. In contrast, Paterson and Pearce (1989, 1992) and Pearce *et al.* (1989) found no effects of regular aversive handling on the growth performance and corticosteroid concentrations in pigs. There is no obvious explanation for this lack of effects in the studies by Paterson and colleagues; however differences between studies in the nature, amount and imposition of handling treatments may be responsible for these apparently contradictory results.

Handlings studies in poultry generally indicate that handling treatments likely to increase the birds' fear of humans may depress growth performance in chickens. For example, in experiments with young chickens, Gross and Siegel (1979, 1980, 1982) found that birds that received frequent human contact, of an apparent positive nature from an early age had improved growth rates and feed efficiency and were more resistant to *Escherichia coli* infection than birds that either received minimal human contact or had been deliberately scared. Barnett *et al.* (1994) found that regular and positive human contact, in comparison to reduced and unexpected human contact, increased fear of humans and reduced egg production in laying hens. The authors speculated that the lower productivity of birds in the latter treatment may have been a consequence of a chronic stress response since there was evidence of immunosuppression in the more fearful birds. Other studies in which positive handling was utilised, have also shown that additional positive handling is associated with increased growth performance in chickens (Thompson, 1976; Jones and Hughes, 1981; Collins and Siegel, 1987). In contrast, Reichmann *et al.* (1978) found no effects of handling on the growth performance of either young broiler or layer chickens, whereas Freeman and Manning (1979) suggested that regular handling decreased growth performance in layer chickens. Since handling may vary from positive to negative in nature for birds, variation in the nature of handling between these studies may have been responsible for the variation in the effects of handling on growth performance.

Handling studies in dairy cattle have shown that aversive handling may depress milk yield in cows (Rushen *et al.*, 1999; Breuer, 2000; Breuer *et al.*, 2003). The results of the study by Rushen *et al.* (1999) implicate the secretion of catecholamines under the influence of the autonomic nervous system affecting milk letdown while the study by Breuer *et al.* (2003) found evidence of chronic stress in negatively-handled heifers. Stressors that result in an acute stress response may depress milk yield due to inhibition of milk letdown (Bruckmaier *et al.*, 1993, 1997; Bruckmaier and Blum, 1998). The long-term stress response of cows and how these responses affect milk yield are poorly understood. One key function of the stress response is to divert food and substrates, such as acetates, glucose and amino acids, away from normal day to day functions such as growth and reproduction (Sapolsky, 1992) and thus during a chronic stress response, the substrates may be diverted elsewhere, thereby interfering with milk synthesis (Breuer *et al.*, 2003). Dam-reared goats, which showed increased avoidance of humans, were found to have greater milk ejection impairment than human-reared goats, suggesting reduced inhibition of milk let-down (Lyons, 1989).

### **ON-FARM RELATIONSHIPS BETWEEN HANDLING AND ANIMAL FEAR, STRESS, PRODUCTIVITY AND HEALTH**

Observations in the Dutch and Australian pig industries have revealed significant relationships, based on farm averages, between fear of humans and reproductive performance pigs (Hemsworth *et al.*, 1981b, 1989). The direction of the relationships indicate that reproductive performance was low at farms where breeding females were highly fearful of humans and the magnitude of these relationships indicate that variation in fear of humans accounted for about 20% of the variation in reproductive performance across the study farms.

Similar negative fear-productivity relationships have been found in the dairy and poultry industries. Significant correlations, based on farm averages, have been found between fear of humans and milk yield in dairy cows (Breuer *et al.*, 2000; Hemsworth *et al.*, 2000; Waiblinger *et al.*, 2002). Negative handling and high fear of humans have also been associated with injuries and poor meat quality in dairy cattle (Lensink *et al.*, 2001b). Studies by Barnett *et al.* (1992), Hemsworth *et al.* (1994b, 1996b) and Cransberg *et al.* (2000) found significant negative relationships, based on farm averages, between the level of fear of humans and egg production in laying hens and efficiency of feed conversion in meat chickens. These studies show that egg production in laying hens and efficiency of feed conversion in meat chickens at farms were inversely related to the level of fear of humans by birds at farms (Barnett *et al.*, 1992; Hemsworth *et al.*, 1994b, 1996). Similarly, in an experiment examining the effects of cage position on fear and egg production in laying hens, level of fear of humans was significantly and negatively related to egg production and efficiency of feed conversion (Hemsworth and Barnett, 1989). In observations on the behavioural response of laying hens to an experimenter, Bredbacka (1988) reported that egg mass production was lower in hens that showed increased avoidance of humans. In poultry, inappropriate fear reactions, like panic or violent escape attempts, can also result in injuries which can lead to infection, chronic pain and debilitation (Jones, 1996, 1997).

Fordyce *et al.* (1988) found that beef cattle that were the most active and vocal when restrained in a weighing stall had the most carcasses bruising and tended to have tougher meat following slaughter. Although part of the behavioural responses of cattle when restrained in a weighing stall would be responses to restraint and novelty, a component of these responses would be specifically to humans. In studying a similar behavioural response to restraint, Burrow (1997) reported that exit speed of beef cattle was negatively correlated with weight gain.

### **EFFECTS OF FEAR OF HUMANS ON ANIMAL WELFARE**

Fear is generally considered an undesirable emotional state of suffering in both humans and animals (Jones and Waddington, 1992) and one of the key recommendations proposed to the United Kingdom Parliament by the Brambell Committee in 1965 (Brambell *et al.*, 1965) was that intensive-housed livestock should be free from fear and there are several reasons why fear of humans will reduce the welfare of farm animals.

Research that has been reviewed in this paper has shown that farm animals that are both highly fearful of humans and in regular contact with humans are likely to experience not only an acute stress response in the presence of humans but also a chronic stress response that is evident even in the absence of humans (Hemsworth and Coleman, 1998). Fearful animals are also more likely to sustain injuries trying to avoid humans during routine inspections and handling. Furthermore, in situations where human contact is aversive, the stockperson's attitude towards the animal is likely to be poor and thus the stockperson's commitment to the surveillance of and the attendance to welfare (and health and production) problems facing the animal may be inadequate. Clearly, fear in farm animals can impact on farm animal welfare and thus this topic of how farm animals are handled is a legitimate welfare consideration.

## REDUCING FEAR IN FARM ANIMALS

Research in the livestock industries indicates that human-animal interactions can markedly limit animal productivity and welfare. Understanding the attitudes and behaviour of stockpeople appears to be the key to manipulating these human-animal interactions to improve animal productivity and welfare.

The sequential relationships between stockperson attitudes and behaviour and animal fear and productivity that have been found in the dairy and pig industries (Hemsworth *et al.*, 1989, 2000; Coleman *et al.*, 1998; Breuer *et al.*, 2000; Waiblinger *et al.*, 2002) demonstrate the opportunities that exist to improve animal productivity and welfare by appropriate selection and training of stockpeople. In fact, studies in these livestock industries have shown that it is possible to improve the attitudes and behaviour of stockpeople and, in turn, reduce the level of fear and improve productivity in commercial cows and pigs (Coleman *et al.*, 2000b; Hemsworth *et al.*, 1994a, 2002). This approach in improving the attitudes and behaviour of stockpeople has been described in detail by Hemsworth and Coleman (1998). Basically, cognitive-behavioural training techniques involve retraining people in terms of their behaviour by firstly targeting both the beliefs that underlie the behaviour (attitude) and the behaviour in question and secondly, maintaining these changed beliefs and behaviour. This process of inducing behavioural change is really a comprehensive procedure in which all of the personal and external factors that are relevant to the behavioural situation are explicitly targeted.

Recent results by Coleman *et al.* (2000a) and Coleman (2001) indicate that job-related characteristics, such as empathy, attitudes towards pigs and towards aspects of work, are useful predictors of work performance of the stockperson and thus, potentially such measures could be assembled into a kit for use in selection of stockpeople in the pig industry. In addition to assisting in selecting stockpeople, assessing the key job-related characteristics of stockpeople may also provide the livestock industries with a good opportunity to monitor the potential impact of individual stockpeople. Screening aids such as attitude and job motivation questionnaires may identify both weakness in individual stockperson and targeted training for these individuals.

## CONCLUSION

While technical skills and knowledge are important attributes of the work performance of stockpeople (Coleman, 2004), two other important but less well recognised characteristics are their attitude and behaviour towards their farm animals. Research has shown that the behaviour of stockpeople can result in farm animals developing fear responses to humans, which can have large motivational and emotional effects on the animals. It is these fear levels, through stress, that may adversely affect animal productivity, health and welfare. While there has been little research conducted on animal health, a limited number of studies indicate the potential impact of human-animal relationships on animal health. Furthermore, stress elicited by fear has implications for animal health because of the close relationship between stress and illness (Moberg, 2000).

In conclusion, there are opportunities to reduce the limitations that human-animal interactions impose on animal productivity, health and welfare. While our understanding of the regulation and impact of human-animal interactions has improved considerably over the last decade or so, recognition of the role of stockpeople on the productivity, health and welfare of livestock has only recently occurred. Appropriate strategies to recruit and train stockpeople in the livestock

industries will be integral in safeguarding the welfare of commercial livestock as well as their health and productivity.

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## PRECISION LIVESTOCK FARMING FOR ANIMAL HEALTH, WELFARE AND PRODUCTION

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### SUMMARY

Precision livestock farming, PLF, is an embryonic technology that applies the principles of process engineering to livestock farming. PLF requires a sensing system for outputs; a mathematical model of input/output relationships; a target and trajectory for controlled processes; and a model-based controller with actuators for process inputs. PLF has great potential to transform livestock production by efficient utilisation of nutrients, early warning of ill health, and reduction in pollutant emissions. While the current focus of PLF should be livestock monitoring, the ultimate goal is to employ PLF as the farmer's aid to automatic management of intensive and extensive livestock production.

**Keywords:** precision livestock farming, monitoring, welfare, health, production, environment

### INTRODUCTION

Precision livestock farming (PLF) is an infant technology that is found in the scientist's and engineer's laboratory; there are few examples – yet – of its routine use on the farm. Its premise is simple: given the many technical, economic and regulatory demands that are complex, exacting and sometimes conflicting, then livestock farming will have to employ automated systems to monitor and manage the main processes involved to remain sustainable. Thus, the European farmer may have to adopt PLF in order to survive in a global market by substituting technology for skilled labour, a trend that has been relentless since modern European agriculture started to develop in the 18<sup>th</sup> century.

This paper reviews the technology of PLF, outlines its potential advantages in European countries (and other developed countries that face the same economic forces), considers some of the hurdles to be cleared by innovative farmers, manufacturers and scientists working together, and suggests how PLF should – and by implication should not – be used in the production of food from livestock. The perspective is that of livestock farming in the U.K.

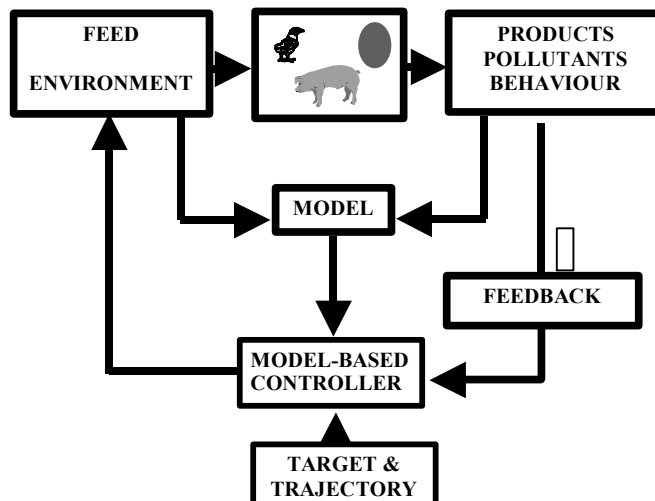
The underlying assumption behind the adoption of PLF is that the interests of both the farmer and the consumer have to be satisfied if PLF is to be useful and be commercially viable. From the farmer's perspective, sustainable livestock production requires tight product specifications to be met profitably by skilled stockmen with minimal adverse environmental impact and a high standard of animal health and welfare; from the consumer's viewpoint, (s)he has only a scant knowledge of livestock farming and its practices, is becoming increasingly concerned about the provenance of his (or her) animal products, and requires his (or her) food to be safe, nutritious and affordable.



In the early days of its development, PLF was also known as integrated management systems but the latter term has fallen by the wayside to ensure that PLF is more closely aligned with precision agriculture (for crop production), its larger cousin. The first conference covering PLF was held in 2001 in Cambridge, U.K. (Wathes et al. 2001) and was noteworthy for the multidisciplinary cast of speakers and delegates. Subsequently, the 1st and 2<sup>nd</sup> European Conferences on PLF (ECPLF) were held in Berlin and Uppsala in 2003 and 2005 respectively (Cox 2003; Cox 2005), while the 3<sup>rd</sup> ECPLF will be held in Skiathos, Greece, in 2007.

### THE BASIC CONCEPTS OF PLF

The definition of precision livestock farming used in this paper is *'the application of the principles and techniques of process engineering to livestock farming to monitor, model and manage animal production'*. Most commonly, closed loop, model-based control systems are used to provide automatic management to meet a specific target. The basic concept of PLF is shown in Figure 1 and is described in detail elsewhere, (e.g. Aerts et al. 2000; Aerts et al. 2003b; Wathes et al. 2005). PLF requires (i) continuous sensing of the process responses (or outputs in the terminology of the process engineer) at an appropriate frequency and scale with information fed back to the process controller; (ii) a compact, mathematical model, which predicts the dynamic responses of each process output to variation of the inputs and can be – and is best – estimated on-line in real time; (iii) a target value and/or trajectory for each process output, e.g. a behavioural pattern, pollutant emission or growth rate; and (iv) actuators and a model-based predictive controller for the process inputs, e.g. feed or the environment.



**Figure 1.** PLF concepts

Although perhaps a semantic point, it seems as if any application of advanced agricultural engineering in livestock farming, e.g. automatic or robotic milking, is considered to be an example of PLF. This is evidently not the case if the formal definition of PLF given above is used.

The essence of PLF is an integrated systems approach to livestock farming with automatic monitoring, modelling and management to drive processes along defined trajectories to meet specified targets. Nor does PLF have to be restricted to saleable products, such as eggs, meat or milk. The concept is general and can be equally well applied to animal behaviour, certain diseases or pollutants, in fact any process that is part and parcel of livestock farming. PLF can also be applied to both extensive and intensive systems of livestock farming.

Implicit in the concept of PLF is the scale at which the approach is to be used, i.e. the unit to be managed. This may be an individual animal, a pen, a building, the animals' bedding or a flock or herd outdoors at pasture. It is quite feasible to use PLF with unidentified individuals; however, the accuracy of their management will be greater if they are identified electronically to allow individual monitoring and management to take place. In general, the finer the scale at which PLF is applied, the greater the expense and the higher the return on the extra capital investment needed to justify the more accurate, finer control of the enterprise. Given the embryonic nature of PLF, there are no studies that address the question of the optimum scale at which PLF should be applied.

### LESSONS TO BE LEARNT FROM CURRENT APPLICATIONS OF PLF

The earliest example of PLF is the Flockman<sup>TM</sup> technology, developed by David Filmer Ltd, U.K., to manage automatically the diet and environment of broilers (Filmer 2001). The key components of Flockman<sup>TM</sup> are: i) real time monitoring of feed intake and live bird weight; ii) novel feed provision using a blend of regular concentrate and whole grain cereals fed in meals; iii) environmental management, including dawn and dusk lighting, integrated with the feeding system; and iv) automatic adjustment of daily feed supply according to deviation of growth from target. Latterly, decision-making in Flockman<sup>TM</sup> was automatic (Stacey et al. 2004), according to real-time measurements of growth rate and food intake. Flockman<sup>TM</sup> was a pioneering example of PLF and, as such, was prone to many of the pitfalls of all new technology. It achieved early success in the U.K. market; approximately 15 percent of U.K. broilers were grown using it several years ago ([www.flockman.com](http://www.flockman.com)), though current usage is thought to be much less.

A similar approach to growing broilers using PLF was taken by Aerts where the objective was to control the growth trajectory of broiler chickens using an adaptive, compact, dynamic process model (Aerts et al. 2003a). Daily food supply was calculated to allow the birds to follow a defined target growth trajectory. Parameters of the growth model, which predicted the response to the control input (food supply), were estimated on-line. This adapted the model to the actual response of weight to feed intake and was the basis for efficient control. The control algorithm developed enabled the broilers to follow different target trajectories with a mean relative error ranging between 3.7 and 6.0%. With a few exceptions, the numerical values of feed conversion ratio and mortality after week 1 were lower and the values of uniformity index were higher in the controlled groups compared with *ad libitum* fed animals.

About the same time as the above developments, a comprehensive research programme to develop PLF to monitor, model and manage the growth of pigs and sows was undertaken by a team of engineers, mathematicians and animal scientists at Silsoe Research Institute and the Universities of Edinburgh and Bristol in the U.K. The PLF system comprised an imaging system for non-invasive monitoring of growth of pigs in a pen (Schofield et al. 1999; White et al. 2004), mathematical models of growth, food intake and carcass composition (Green & Whittemore 2003; Green & Whittemore 2005), and control of feed supply according to group or individual

requirements (Parsons et al. 2007). The novelty of image analysis to monitor growth meant that the animals' shape and size could be measured directly, as well as their weight (Doeschl-Wilson et al. 2004; Whittemore & Schofield 2000), which was estimated from their plan area. The system was tested on a semi-commercial scale but has not been taken to the market.

The initial applications of PLF have been the growth of housed pigs and poultry though, in principle, the PLF approach could be applied to any farmed species, including those animals farmed extensively. There are a number of lessons to be learnt from these pioneering attempts by a few groups to develop PLF. Given the importance of the efficient management of growth and feed, the difficulties and tedium of manual weighing and the need to match nutrient supply to demand, it is not unsurprising that growth was the initial focus. Needless to say, the commercial success of these applications has been poor. The reasons for this are several folds. Firstly, the poor profitability of pig and poultry farming has inhibited capital investment on unproven new technology, even when the expected payback period is only several years. Secondly, much if not all of the development work that should have been carried out and paid for by the technology developers was not done, in which case the first customers (unwittingly) acted as guinea pigs to identify and resolve any shortfalls or problems in the technology. Thirdly, the sophistication of the computer hardware was too great for the skills and knowledge of many stockmen; in integrated operations, e.g. broiler farms; management decisions on feed formulation and supply (according to target) are not made by the stockman but instead by the production director/manager.

The penalties of early adoption of a new technology can be severe if the researcher's promises are not met on the farm. The new technology rightly acquires a poor reputation, making it harder for the researchers and commercial manufacturers to secure sufficient funds to overcome the outstanding technological problems or to market it to sceptical farmers. In the light of these lessons, it therefore seems timely to reappraise the technical and commercial prospects for PLF.

### **THE ELECTRONIC STOCKMAN – AN ENGINEER'S PIPEDREAM OR A GENUINE PROSPECT FOR THE FUTURE?**

Electronic monitoring of livestock is at the heart of PLF. Currently the major examples of electronic monitoring of livestock are identification of cows, sows and sheep using RFID tags, detection of oestrus, and measurement of milk yield and composition in dairy herds. The most widespread use of agricultural electronics is to control the thermal environment of housed pigs and poultry where sensors for air temperature, relative humidity and, in some cases, ventilation rate, are integrated in controllers. These applications have been extremely successful in helping a farmer to manage his herds or flocks but the question remains why has not more use been made of electronics to monitor livestock?

In the mid 1990s, Frost forecast that automatic monitoring systems for farm animals would be developed soon that would integrate information from multiple sensors with mathematical models and knowledge bases to aid the farmer in decision-making, i.e. the basics of PLF (Frost et al. 1997). Even then, there was an abundance of sensors that were potentially available for electronic monitoring, e.g. of animal weight, behaviour, and physiological parameters using acoustic, chemical, gravimetric and other sensors. Frost concluded that "monitoring and control in livestock production is relatively undeveloped compared to most major industries. This is largely because most of the factors to be monitored are biological and inherently variable and unpredictable". Ten years after this review, are these conclusions still correct?

European agricultural engineers, in association with leading agricultural engineering companies such as Fancom, De Laval and Petersime, have organised a series of biennial workshops (SMART) since 2000 to oversee the development of monitoring systems for livestock. At the most recent workshop (SMART 2006, Italy; [www.smart2006.eu](http://www.smart2006.eu)), papers on the following processes were presented; most of the work described was at the experimental stage:

<u>Cattle</u>	<u>Pigs</u>	<u>Poultry</u>
Rumen pH, Blood & Milk fat	Vocalisations & Activity	Birds: Liveweight
Temperature: rumen & vulva	Farrowing behaviour	Eggs: Temperature, albumen,
Lameness & Location	Growth & Body composition	Ph, Nitric oxide release
Calving behaviour		

Clearly, there has been significant investment in research in Europe on the use of sensors and sensing systems over the past decade and more. However, most reports of sensors and sensing systems for livestock refer to their use in experimental situations with few dealing with commercial use on livestock farms. Electronic monitoring of livestock is uncommon on commercial farms for three principal reasons.

- i. Most research on electronic monitoring does not involve manufacturing companies from the start, with clear specifications set for commercial success in terms of demand, performance and manufacturing feasibility.
- ii. While technical success can be shown under idealised conditions with a few animals in an experimental setting, complete sensing systems do not undergo proving trials on a large scale under semi-commercial conditions, with full-scale demonstration of the proven technology to farmers, consultants and journalists.
- iii. The demand by livestock farmers for new monitoring technologies has either not been assessed or, if such a market analysis has been carried out, the results are not widely known within the research community.

It is only once these commercial weaknesses have been addressed that electronic monitoring will realise its potential. Such success would eventually allow PLF to be considered properly by farmers.

### **THE PROSPECTS FOR PLF – THE NEED FOR AUTOMATIC MONITORING IN LIVESTOCK FARMING**

The commercial climate in which European livestock farmers have to operate has changed markedly over the past decade. From a position of strength in which many sectors of livestock farming were ‘price setters’, livestock farmers, with a few exceptions such as organic farmers or niche suppliers, are now ‘price takers’. Over the past decade, the net farm income on farms has fluctuated widely, reflecting substantial changes in the profitability of livestock farming in the U.K (<http://statistics.defra.gov.uk/esg/publications/auk/2005/2-5.xls>). Furthermore, the regulatory burden has increased, particularly in terms of the allowable environmental impact of livestock farming due to the introduction of the EU’s Integrated Pollution Prevention and Control directive. Concurrently, attention to the acceptable standard of animal welfare has led to regular inspection of livestock farms by veterinarians and others. Overall, livestock farmers effectively require a number of explicit or implicit permits to operate in order to satisfy the consumer that his or her

food is safe, traceable and produced within Government guidelines for environmental impact, welfare and nutritious value. Many of these demands on livestock farming can be met by a farm assurance scheme with independent audit of claims, and labelling at the point of sale to inform the interested purchaser about the environmental, nutritional, or welfare provenance of animal-based food (or other products).

This analysis suggests that the emphasis of researchers and commercial developers over the next decade should be on the use of engineering technology to monitor livestock farming with management decisions left to the farmer, perhaps aided by a full PLF system. The ever-lower costs of technology should be harnessed to satisfy the demand for information about animal-based products and farming methods, thereby meeting a current need that should be much simpler to achieve than in PLF.

Experience over the last decade therefore shows that although there is no shortage of engineers clamouring to develop a large armoury of sensors and sensing systems that could be used to monitor livestock farming, suitable applications of the highest priority have rarely been identified. In this sense, agricultural engineers have failed society. Historically the main influence on the suitability of an application of livestock monitoring has been its potential impact on profitability, but increasingly the need to demonstrate regulatory compliance and/or provide consumer assurance will be at least, if not more, important.

An obvious example of a product that should sell well to livestock farmers is an automatic weigher for pigs or broiler chickens and yet, although various technologies have been developed and marketed, (e.g. Schofield et al. 1999; Turner et al. 1984)), automatic weighers are not in common use. Since pig and poultry farmers are paid by live weight, then a means to determine the efficient conversion of animal feed into saleable meat should be essential if the processor's strict requirements are to be met. The reasons for this are not well understood but could include the ability of the expert farmer to estimate weight by eye or the poor reliability of automatic weighers. This apparent failure to monitor perhaps the most important determinant of profitability is astonishing. It is as if Henry Ford calculated the performance of his factories by counting the number of Model T automobiles in the dealer's showroom or scrap yard.

Automatic monitoring in livestock farming should therefore be developed for environmental emissions, zoonoses, organoleptic properties of meat, and welfare since it is these credence characteristics of animal products that are valued by the consumer, and hence are of value to the producer. After all, if the farmer cannot guarantee to the consumer, processor or the regulatory authorities that his animals have been produced to their specifications and satisfaction then his animals will be unmarketable. Suggestions for suitable processes in livestock farming to be monitored are given in Table 1.

**Table 1.** Processes in livestock farming that are suitable for automatic monitoring

<b>On the farm:</b>	<b>Reasons for monitoring</b>
Emissions of ammonia, methane and carbon dioxide from livestock buildings	Legislation, e.g. IPPC directive
Presence of salmonella, campylobacter and other zoonoses in pigs, cattle, broilers and laying hens, as appropriate	Legislation on food safety
Predictive markers of meat quality	Consumer demand
<b>During transport and at the abattoir:</b>	
Welfare	Consumer demand & legislation
Meat texture and tenderness	Consumer demand

The primary justification for monitoring these processes is either legislation or consumer demand. If monitoring was widespread, then the information could be used as part of a national surveillance scheme for environmental emissions, animal welfare or zoonoses. For example, there is much interest at present in the development of indicators of welfare so that consumers and regulators can be assured that their demands are being satisfied or that there is compliance with the legislation, respectively. Once the basis of a scheme for surveillance monitoring of welfare has been developed, then it will be feasible to determine whether improvements are being made. Discussion of welfare indicators in general is outwith this paper's remit, but is quite apposite in the context of welfare during transport or while the animals are at the abattoir. Monitoring welfare in the lairage and abattoir could be as straightforward as continuously measuring levels of mechanical and animal noise, the number of cattle or sheep that baulk in a race or require a second stun (perhaps using image analysis), the electrical current or gas concentration experienced by poultry during stunning, the dirtiness of the hide or fleece, or the prevalence of hock burn in broiler chickens. All of these are indicators of welfare at various stages in the animal's life and would require limited research and development before use.

Undoubtedly there are technical difficulties to be overcome in developing sensing systems for livestock monitoring. The requirement for low cost may be met by using sensors developed for other industries, e.g. cameras in mobile phones. Deployment of a sensing system will produce questions relating to the number and location of sensors, and their robustness, reliability and data transfer. However, perhaps the most difficult challenge will arise when the data are analysed and interpreted. How will the key findings be communicated to the farmer, consumer and regulator? Finally, successful commercialisation will require researchers to work closely with manufacturing companies to avoid the problems highlighted earlier. Given formation of a suitable partnership then a monitoring system for any one of the processes listed in Table 1 could be marketed within three to five years.

## CONCLUSIONS

Some elements of PLF are already commonplace on livestock farms, i.e. sensing systems for milk yield in dairying, and their use should be part of livestock production irrespective of the greater potential of PLF to manage livestock automatically. If the promise of PLF is to be realised then three barriers need to be overcome before commercial uptake occurs: i) PLF technology needs to be developed that is based upon robust, low cost sensing systems and data-based models with meaningful parameters that enable control of two or more interacting physical and/or biological processes; ii) appropriate applications must be identified with targets and trajectories specified for the main candidate processes; and iii) development and demonstration must be completed at a commercial scale to demonstrate that any investment will have a reasonable return and that the technology is reliable. Given the scale of these challenges and the timescale needed to overcome them, then current effort should focus on the development of monitoring systems for livestock that satisfy the demands of consumers and regulators for safe, nutritious food produced from farm animals of guaranteed standard of welfare within acceptable limits of environmental emissions.

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## **PARALLEL SESSIONS B1; B2, C; D**

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### **B1 – ANIMAL HEALTH – ECONOMICAL ASPECTS IN ANIMAL HUSBANDRY**





## ORAL PRESENTATIONS

### CAN WE DECREASE THE INCIDENCE OF PERINATAL MORTALITY IN THE COW?

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#### SUMMARY

During the last decades there has been a trend of increasing rates of stillbirths (death of a mature calf foetus during calving and the first 24 h pp) especially in Holstein Friesian heifers. The cause of stillbirth with a non-infectious aetiology is likely to be multifactorial, but the majority of stillbirth might be caused by direct and indirect asphyxia. In practice it is generally not possible to measure the acid-base metabolism of foetuses therefore it is very important to know those changes which may occur during normal calving and obstetrical assistance in order to be able to decrease perinatal mortality.

**Keywords:** perinatal mortality, newborn calves, acid-base disturbance, acidosis

The profitability of cattle breeding is greatly influenced by the rate of calves being born alive and reared to adulthood. In spite of the speedy developments of animal breeding, perinatal mortality is still very high (4 to 7%) and constitutes approximately half of the total calf losses (Anderson and Bellows, 1967; Voelker, 1967; Szenci and B.Kiss, 1982; Mee, 1991). Perinatal mortality (stillbirth) is defined as the death of mature calf foetuses during calving or in the first 24 h of postnatal life.

During the last decades there is a trend of increasing rates of stillbirths especially in Holstein Friesian (HF) heifers. In the Swedish HF-heifer population stillbirth rate has increased from 4% to 11% (Gustafsson et al., 2004). As other authors found, at first calving around 10% of the calves are born dead or die on the first day (Berlgund et al., 2003; Meyer et al., 2000; Steinbock et al., 2003). In the Netherlands stillbirth rate for heifers was reported to be 12,2% in 1999, and in the USA it was 13,2% in 1996 (Steinbock et al., 2003).

The incidence rate of stillbirths (294 out of 4103) in a large-scale Hungarian Holstein-Friesian dairy farm between 1973 and 1978 was 8,3% for heifer calvings and 6,5% for cow calvings, respectively (Szenci et al., 1981). In the same farm the stillbirth rate was 8,7% for heifer calvings and 5,9% for cow calvings in 2003, respectively. All together 124 of 1733 newborn calves were lost (7,2%) in the perinatal period. Comparing the two periods there were no increase in perinatal mortality, however it is still very high. These figures call attention to the importance of examining the causal factors of perinatal mortality.

The cause of stillbirth with a non-infectious aetiology is likely to be multifactorial but the majority of calves might die due to direct and indirect asphyxia because in 73 to 75% of the calves that died in the perinatal period no pathological changes were detected (Hahnsdorf, 1967;

Greene, 1979). In a recent study, occurrence of asphyxia in calves dying perinatally was 58.3% (Schuijt, 1990).

As a result of disturbances in the uteroplacental circulation occurring during parturition due to the rupture of foetal membranes and uterine contractions, all calf foetuses develop more or less severe hypoxia and consequently acidosis. The foetus responds to hypoxia by an oxygen-conserving adaptation of its circulation. This means that all organs, that are not essential for intra-uterine life (lungs, spleen, thymus, muscles and skin, gastrointestinal tract, possible also the liver and kidneys) are supplied with minimum blood. Oxygen is spared for essential organs like the brain, heart and adrenal glands. Increased heart rate (tachycardia), blood pressure and blood flow are also observed. In the case of relatively high hypoxaemia the frequency of heart activity decreases (bradycardia). Circulation changes and reduced oxygen consumption result in oxygen tension in the blood being maintained within physiological limits for some time. This oxygen-conserving adaptation, however, means anaerobic glycolysis in all tissues with minimum blood supply. Under normoxic conditions glucose as the main energy source is reduced to pyruvate via the citric-acid cycle. The first step to pyruvate is anaerobic, the second step consists of oxidation of pyruvate via the citric-acid cycle to the final products, CO<sub>2</sub> and H<sub>2</sub>O. During oxygen shortage glucose can only be metabolised anaerobically to pyruvate, which is mostly reduced to lactic acid. Energy output in this process is small, but it is sufficient to maintain metabolism for some time. Anaerobic glycolysis, however, has a great disadvantage because energy production is reduced, the carbohydrate reserves are rapidly exhausted and metabolic acidosis develops by accumulation of acid metabolites (lactic acid). At birth all foetuses therefore suffer from a respiratory as well as metabolic acidosis. It is the degree of acidosis that finally determines whether the foetus lives or dies. Vital cell functions cannot take place in severe acidosis, at a blood pH value of 6.7 foetal life ends. Before that, the organism's regulatory system of chemical buffering in the blood comes into operation to keep the offspring alive. Bicarbonate is the most important buffer. The others are haemoglobin, plasma proteins and phosphate buffers (Walser and Mauer-Schweizer, 1978; Eigenmann et al., 1983a; Szenci, 1984; Grunert et al., 1985).

The duration of survivable asphyxia always depends on the reserves of glycogen in the heart muscle (Dawes, 1968). The surviving period for calves with induced anoxia is between 4 to 6 minutes: four of 6 foetuses subjected to 4 minutes of anoxia survived whereas all others died when the umbilical cord was clamped for 6 or 8 minutes (Dufty and Sloss, 1977).

The degree of asphyxia can be evaluated by measuring the acid-base parameters in the blood. These parameters are as follows: pH, pCO<sub>2</sub>, pO<sub>2</sub>, base excess (BE: directly expressing the quantity of base /minus/ or acid /plus/ required restore the acid-base balance), actual bicarbonate (HCO<sub>3</sub>: the titratable hydrogen carbonate content of whole blood) (Szenci and Nyirő, 1981).

A field study was performed in a large-scale Hungarian dairy herd (Dutch Friesian/Holstein Friesian crosses) where perinatal mortality ranged previously between 7.4% and 8.6% (Szenci et al., 1988). Delivery of 58 calves, all in normal anterior presentation, was uncomplicated; 3–4 attendants assisted with traction and delivery was completed within 30 minutes after the appearance and rupture of foetal membranes. The duration of traction was measured. In another study Caesarean sections of 44 black and white and red and white Dutch Friesian cows were performed within 4 to 5 hours after spontaneous rupture of foetal membranes in each case (Szenci and Taverne, 1988).

Foetal blood samples were collected by puncturing the *v. metacarpalis volaris superficialis* before the onset of traction and by puncturing *a. or v. umbilicalis* before the onset of extraction during Caesarean section.

Before and after birth, calves were assigned according their pH values to one of three groups as suggested by Eigenmann et al. (1981):

Group 1 blood pH > 7.2 (normal /physiological acidosis/= slight, combined respiratory and metabolic acidosis)

Group 2: blood pH 7.2–7.0 (acidotic = moderate, combined respiratory and metabolic acidosis)

Group 3: blood pH < 7.0 (severely acidotic = severe, combined respiratory and metabolic acidosis)

Shortly before the onset of traction, 25 out of 58 calves, or 25.8%, had moderate or severe acidosis (Szenci et al. 1988). In case of Caesarean section, 16 of 44 calves, or 36.4% had moderate or severe acidosis before the onset of the extraction from the uterus (Szenci and Taverne, 1988). The death of 2 newborn calves that were seemingly normal before birth indicates that either forced traction lasted too long or another, non-diagnosed complication occurred, such as premature compression and/or rupture of the umbilical cord, or premature separation of the placenta (Szenci et al., 1988). In agreement with Eigenmann (1981) and Schuijt (1990) forced traction jeopardises the calf's survival. Of the 4 calves (acidotic) only 1 was alive after birth. The intra-uterine pH of the 3 other calves, which were stillborn was nearly 7.0. This intra-uterine acid-base changes before traction or Caesarean section may be explained by late detection of the first stage of labour or by placental and/or umbilical cord anomalies which are responsible for disturbance of the transplacental gas exchange during calving (Szenci et al., 1988).

On the other hand, Held et al. (1985), using prenatal base-excess as a criterion in a study of 217 calvings, found 57.6% of the calves normal (BE > -6.0 mmol/L), 24.9% acidotic (BE: -6.0 and -12.9 mmol/L), and 17.5% severely acidotic (BE ≤ -13 mmol/L). Perinatal mortality measured 0%, 8% and 51% in these three groups, respectively. The calves were delivered either spontaneously or aided by moderate pulling or by Caesarean section. In grouping of our calves delivered by Caesarean section according to Held et al. (1985), a very similar result (normal: 59.1% and acidotic: 40.9%) could be detected, which indicates that both parameters (pH and/or BE) are suitable for grouping of newborn calves (Szenci and Taverne, 1988).

Close correlation was also reported between the duration of the dilatation stage and the number of severely acidotic calves (less than 2 hours: 0%, 2 to 4 hours: 19%, 4 to 7 hours: 44%) by Held (1983).

Eigenmann et al. (1981) clearly showed that the prognosis for calves with intra-uterine pH < 7.2 is very poor, even when they are delivered by Caesarean section; 56% of these calves died during the first week. Although our data are perhaps less dramatic – of the calves with a prenatal pH lower than 7.2, after traction 33% and after Caesarean section 12% died within two days – both studies strongly indicate that the intra-uterine oxygenation and acid-base balance of acidotic calves should be improved before further obstetrical interference takes place. If this can not be performed the obstetrician may be supported by prenatal acid-base analysis in choosing the type of assistance (traction or Caesarean section) since the latter can be a careful procedure for blood gas and acid-base status of the newborn calves (Massip, 1980a; Eigenmann et al., 1981; Szenci, 1985a,b).

According to these results it was revealed that besides disturbances of diaplacental circulation except of the abnormality of umbilical cord and foetal membranes it is the duration of expulsive period (Held, 1983), the way of assistance (Eigenmann, 1981; Eigenmann et al., 1981; Szenci, 1983 and 1985a,b) and the duration of traction (Szenci, 1983; Szenci 1985a; Szenci et al., 1988; Schuijt, 1992) which influences the acid-base balance of newborn calves. At the same time maximum and relative force of traction appeared to be very weakly correlated with the changes of blood gas and acid-base values in calves during extraction. The highest relative force of traction

during uncomplicated extractions was 5.4 kg per kg birth weight (Schuijt, 1992). In the case of traction it is the duration of passage through the birth canal and within this period – the duration of the compression of the umbilical cord which may play a decisive role. Therefore in the case of a complicated calving, the mode of assistance should be chosen by taking into consideration the economic point of view, as well as the aim that acid-base balance of newborn calves should be disturbed as little as possible that asphyxia should be avoided. If it is not possible the adequate treatment of asphyxiated newborn calves must be solved (Eigenmann et al., 1983a; Szenci, 1985).

### IN CONCLUSION

At present, in veterinary practice the main emphasis should be paid on the prevention of asphyxia of calves at birth, since instruments suitable for a reliable clearing of respiratory passages and for the maintenance of this state, and for artificial respiration of calves under practical conditions are not yet widely used. The most important breeding objectives are to reduce the number of calving assistance required. This is even more important, since calving assistance in itself may result in a shift of the calf's acid-base balance. In case of dystocia, the mode and time of calving assistance should be chosen with regard to profitability factors and in a manner which would allow the least possible shift of the calf's acid-base balance towards acidosis (Held, 1983; Szenci, 1983 and 1985a). Before applying traction, the measurements of the soft birth canal should always be considered when dilatation of the soft maternal passages is not sufficient they must be expanded non-surgically or surgically (episiotomia lateralis) and obstetric lubricants should be used to avoid tractions longer than 2 to 3 minutes (Szenci, 1985b) and rib and vertebral fractures due to excessive traction (Schuijt, 1990). If a prolonged traction is expected, Caesarean section should be carried out to save the calf and to prevent injuries to the maternal birth canal. Recent studies have shown that before making a decision as to the mode of calving assistance in an animal hospital, the results of acid-base balance measurements from blood samples should be considered. The routine use of complex treatment of calves born with severe asphyxia may reduce the postnatal calf losses (Szenci, 2003). In addition to an adequate therapy (Eigenmann et al., 1983a), particular attention in the case of calves with asphyxia should be paid to the ingestion of sufficient amounts of colostrum, since the lack of colostrum uptake is accompanied by an increased susceptibility to *E. coli* infections (Eigenmann et al., 1983b; Besser et al., 1990).

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## ESTIMATION OF ECONOMIC LOSSES ON NEMATODE INFESTATION IN GOATS IN SRI LANKA

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### ABSTRACT

Goat husbandry is one of the main income generating sources in livestock farming population in Sri Lanka. It is high income enterprise with minimal inputs especially in dry zone of Sri Lanka. Recent livestock development program have highlighted the potential of goat rearing as an alternative to meet the short fall of meat supply in the country. Nematode infestation is an important parasitic problem and gives substantial losses in goats in Sri Lankan farms. It was reported that the gastro intestinal nematode infestation was a common condition in kids (89%), young goats (94%) and adult goats (84%). Further, it was estimated that 14 percent of the total cost of the government goat farm was spent control of nematode infestation. In this study the economic losses on nematode infestation in goat industry in Sri Lanka in year 2004 were estimated using previous research findings and the available data. The main direct economic loss was the losses in weight gain and it was estimated as 170 million rupees per year. Economic losses due to mortality in kids are estimated as 59 million rupees per year. A total of about 230 million rupees are lost per annum and it is much more higher when account the other direct losses on common signs as reduction of milk production, low kidding index, high return rate, high rate of abortions and still births in infected goats. Additionally cost on veterinary services and drugs, decreasing quality in animal product such as carcasses and hides, some residual nervous signs, resistance against broad spectrum antihelmintic groups' synergies the economic losses on nematode infestation. In conclusion nematode infestation causes over 230 million rupees loss in Sri Lankan livestock sector and in need of immediate attention.

**Keywords:** goats, nematodes, economic losses

### INTRODUCTION

Goat husbandry is one of the main income generating sources in livestock farming population in Sri Lanka. It is high income enterprise with minimal inputs especially in dry zone of Sri Lanka. Recent livestock development program have highlighted the potential of goat rearing as an alternative to meet the short fall of meat supply in the country. About 75 percent of the meat goat population is concentrated in dry and dry intermediate zones of Sri Lanka (C&S, 2004). Studies on birth weight revealed that the average birth weight was 2.69 kg for Kottukachchiya breed (Premasundara *et al*, 1993) and that of single born Jamnapari (J), Kottukachchiya and their crosses (KJ) are 3.2 kg, 2.18 kg, 2.65 kg respectively (Premasundara *et al*, 1993). Further, it was

recorded that the average birth weight of Betal goat was 3.48 kg in state farms (Jayaruban, 1992). Premasundara *et al*, (1993) has reported that the range of mature body weights of Kottukachchiya breed females and males are 35–40 and 40–50 kg respectively at Kottukachchiya farm and mean total of milk production during the first 40 days were 15.3, liters. But the analysis of data on birth weights and mature weights of goats in small scale farms at field level shows that the average birth weight was around 1.5 kg and the body weight of adult male was around 25 kg and adult female was 20 kg (DAPH, 2004) and the average milk production was around 10 liters for first 40 days in dry zone of Sri Lanka. Therefore, it is clear that the weight gain and milk production in goats at field level was lower than in the state farm condition. The factors viz. genetic, management (feeding) and diseases are the main causes for low production performance. Nematode infestation is an important disease condition that causes economic losses in goat industry in the country. It was estimated that 14 percent of the total cost of the government goat farm was spent control of nematode infestation (Jayasinghe, 1993).

The economic importance varies between countries and region depending on climate and intensiveness of farming in the area. However losses due to parasitic diseases are greater in tropical countries than in temperate countries because the parasites are much more numerous and abundant in tropical environment. This is mainly because of the higher temperatures in the tropics that favor the rapid multiplication and propagation of the free living stages which are the mainly responsible for the dissemination of the parasites. There are several types of nematodes in gastrointestinal tract, respiratory tract and central nervous system in goats in Sri Lanka (Seneviratne, 1955, Senadeera, 1967, Faizal, 1995). It was reported that gastro intestinal nematode infestation was a common condition in kids (89%), young goats (94%) and adult goats (84%) and causes losses in weight gain (Faizal *et al*, 1999). Therefore, this study is an attempt to estimate the economic losses on nematode infestation in Sri Lanka using available statistics and previous findings.

## MATERIALS AND METHODS

### Sources of data

#### Livestock statistics

Livestock statistics on goat population and slaughter were obtained from the Department of Census and Statistics (2004), Sri Lanka. The farm gate goat price of live animals was taken from MLD & EI data (2004).

#### Incidence rate

The incidence of nematode infestation was calculated using previous publications (Welgama *et al*, 1975, Fizal and Jayasinghe, 1996, Fizal *et al*, 1997, Fizal *et al*, 1999). It was reported that there is an influence of rain fall in the presence of larvae in pasture and young goats are more susceptible for parasitism. The presence of *Heamonchus contortus*, *Ostertagis spp*, *Trichostrongylus spp* and *Oesphagostomum spp* were 61.5%,6.3%,16.9%,9.3% respectively in untreated faecal samples in goats. Gastro intestinal nematode infestation was a common condition in kids (89%), young goats (94%) and adult goats (84%) and causes losses in weight gain (Faizal *et al*, 1999). Therefore, the prevalence rate was taken as 70% in this study.



### Production and reproduction parameters

Previous research on production and reproduction aspects findings and the data used in estimation is given below.

1. Kidding rate varies with the breed reared in the farm. Although the year round kidding took place, the frequency of kidding showed bimodal pattern with a peak of (72%) during December to March. Boer gave the highest twinning rate(38–50%) and Boar cross gave the lowest twinning rate(3%) with a mean litter size of  $1.13 \pm 0.12$  (Jayaruban and Weerasekara, 1993). The kidding rate was taken as 1 per year in this study.
2. Kidding interval was reported as the 304,309 and 321 days for Beetal, Boar crosses and Jamnapari respectively (Jayaruban and Weerasekara, 1993). Kidding interval was taken as one year (365 days) in the estimation.
3. Premasundara *et al*, (1993) has reported that the range of mature body weights of Kottukachchiya breed females and males are 35–40 and 40–50 kg respectively at Kottukachchiya farm. Body weight of adult animal was 36.34 kg, 36.20 kg when the population size was 60 and 120 respectively in goat herd in dry zone of Sri Lanka (Jayaruban and Zuhry, 1998). The body weight was taken as the 35 kg of an adult animal.
4. Kid mortality rate due to parasitism and in dry zone goat farms in Sri Lanka studied and reported as 15% (Seneviratne, 1964) and 27% (Wijewardna, 1992). The kid mortality due to nematode infestation was assumed as 20%.
5. Weight loss is the major loss which is difficult to estimate on parasitic infestation. The cause of anathematic treatment in rainy season increased the body weight by 37% according to the study of Fizal *et al*, (2001). It was taken the weight loss as 37% in this study.
6. The farm gate of an adult animal varied from Rs.4000–1650 and of weaned kid from Rs.1250.00 – Rs. 1650 (MRD & EI, 2004). The price was taken as Rs. 3000.00 per adult animal and Rs.1500 per weaned kid in the estimation.
7. Annual off take of the animals was assumed as 20% of the female population and 50% of the male population (DAPH, 2004).

### Analytical methods

The major losses were divided in to two main sources viz. direct losses and indirect losses. The apparent losses such as deaths and insidious losses such as loss of weight, infertility, low kidding index, high rate of abortions and still births in infected goats etc were taken as the direct losses. The losses due to expenses on veterinary drugs, quarantine measures, etc were taken as the indirect losses. The direct losses were estimated using above assumptions. The loss of fertility could not estimate due to absence of research findings on that aspect.

#### Direct losses

**Loss of kids (Mortality) was estimated using following formula**

#### Economic losses on kid mortality

No. of kids born per year = Female breed able goat population X kidding rate .....(1)

No. of deaths of kids per year = (1) X mortality rate.....(2)

Estimated losses per year = (2) X Average cost per kid per year .....(3)

**Loss of weight****Economic losses on weight loss was estimated using following formula**

Total weight of animals = Goat population X average weight of the animal....(4)

Weight loss per year = (4) X take off X weight loss .....(5)

Estimated losses per year (Rs) = (5) X average price per live weight kg ... (6)

Weight loss per year = (7) X off take X weight loss .....(7)

Estimated losses per year (Rs) = (8) X average price per live weight kg .....(8)

Total loss per year (Rs) = (3) + (8)

**Indirect losses**

Cost on veterinary services and drugs, decreasing quality in animal product such as carcasses and hides, some residual nervous signs, resistance against broad spectrum antihelmintic groups (Jayasinghe,1996) synergies the economic losses on nematode infestation.

**RESULTS AND DISCUSSION**

The main losses viz. kid mortality and weight loss were calculated using above assumptions and data sources for the year 2004 and presented in the table 1.

**Table 1.** Estimated cost on nematode infestation in goats per year(2004)

Type of loss	Quantity	Cos Cost (Millions)
		SLR
1.Kid mortality	39547.4 kids	59.32
2.Weight loss (adults)	1417.94 MT	170.15
<b>Total loss</b>		<b>229.48</b>

The loss of kids was estimated as 39547.4 animals per year due to nematode infestation. It was 59.32 million Sri Lankan rupees (SLR) per year in monetary value (Table 1). The table 1 shows about 1417.94 MT of mutton has been lost due to nematode infestation in Sri Lanka. It was 170.15 million in Sri Lankan rupees (SLR). The total loss was estimated as 229.48 million Sri Lankan rupees in monetary value. The value should be much more higher when incorporate the cost of infertility, milk loss and veterinary charges. Development of resistance to some anthelmintics complicates the problem and increases the cost.

**CONCLUSION**

Sri Lankan livestock industry losses a large amount of currency due to nematode infestation annually. Immediate attention is needed in feeding management and prophylactic treatments to overcome the problem.

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## MODELING THE CAUSES OF LEG DISORDERS IN FINISHER HERDS

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### SUMMARY

We present a probabilistic model that estimates the probability distributions of different manageable causes of leg disorders in finisher herds. The purpose of the model is to identify the probability distributions of three cause-categories of leg disorders: Infectious, Inherited and Environmental. The probabilistic model is constructed using an Object-Oriented Bayesian Network and the parameters in the model are based on published literature and expert opinions. The objects of the model are instances of two classes (herd -and pig class), each contributing with information to the model. The probability distributions can serve as a consistent set of inputs for economic calculations.

**Keywords:** object-oriented Bayesian Network; finisher herds; leg disorders; economics

### INTRODUCTION

Leg disorders in finisher herds cause economical losses for the farmer. The losses are due to reduced productivity (e.g. decreased daily weight gain), medical treatment costs and increased work load due to the physical handling of the pigs. Furthermore, leg disorders are also an indication of poor animal welfare. Leg disorders in finishers can conveniently be divided into three major cause-categories: Infectious, Inherited and Environmental. Infectious leg disorders represent arthritis caused by infectious agents such as *Mycoplasma hyosynoviae*, *Erysipelothrix rhusiopathiae*, *Haemophilus parasuis* and *Streptococcus sp.* Osteochondrosis can be characterized as an inherited leg disorder and is a disturbance in the endochondral ossification of the cartilage and bone (Grøndalen, 1974). Environmental leg disorders represent injuries to the limb and claw such as fractures and claw lesions.

Control strategies against leg disorders will depend on the cause-category. Thus, antibiotic treatment will be used against infectious arthritis whereas improvement in the pen and floor construction will be the strategy against environmental leg disorders. In order to implement the optimal control strategy for leg disorders in a herd it is essential to know the most prevalent cause-category. The purpose of this paper is to create a probabilistic model that can estimate the probability distributions of different manageable causes of leg disorders in finisher herds.

### METHOD

#### Object-Oriented Bayesian Network

The probabilistic model for leg disorders is constructed using an Object-Oriented Bayesian Network. In this model the objects are instances of two classes: the herd class and the pig class.

The object is the basic component in the object-oriented paradigm and each object has entities with identities, states and behaviour. The two classes represent objects that share the same structure, behaviour and attributes (Bangsø, 2004). The herd class has one object with a number of entities (e.g. the stocking density of pens and the herd size). The pig class is used to create several objects and each object has entities representing animal specific information (e.g. clinical signs of lameness, gender and results from diagnostic tests). The object-oriented paradigm is included in the framework of a Bayesian network. Hence, the model is a static model for a single herd and all interdependencies are described using conditional probability distributions. The model is a directed acyclic graph where the directions of the links represent the biological causalities. The Bayesian network allows information to flow in the opposite direction of the causality (Jensen, 2001). Each node in the model is discrete and represents a finite number of states. Input to the model is the state of the various risk factors or diagnostic tests. A few nodes in the model are latent nodes which are not directly observable but help in the specification of the model. The major outputs of the model are the pressure of the three cause-categories of lameness at herd level. These nodes are considered to be continuous, however, due to properties of Bayesian networks we make a discretization of the nodes.

### **The structure of the biological model**

The background for the qualitative structure of the model is based on evidence from the literature as well as information from experts (literature references are not included in this paper). For the herd class, evidence regarding the nodes: *Production* (sectioned or continues production), *Purchase* (number of suppliers) and *Herd size* (number of pigs slaughtered annually) will influence the probability distribution for infectious arthritis. However, the nodes: *Floor* (floor type in the pen), *Straw* (use of bedding) and *PenDen* (stocking density in the pen) will influence the probabilities for all three cause-categories. The breed as well as the weight gain will affect the occurrence of inherited leg disorders.

It is the intention that a number of pigs in the herd can be selected randomly and observed for clinical signs of lameness. Evidence on whether or not the selected pig shows clinical signs of lameness is included in the node *ObsLame*. The true state of lameness for the individual pig is presented in the latent node *PigLame* and the relation between the two nodes: *PigLame* and *ObsLame* depend on the sensitivity and specificity of the clinical observation. Based on diagnostic tests (e.g. pathological and bacteriological tests) as well as further information regarding the individual pig (e.g. tail bite, gender and lean meat percentage) it is possible to estimate the probability distributions of the specific lameness diagnoses. Hence, each object in the pig class will provide evidence regarding clinical signs of lameness, and for the lame pigs it is further possible to specify the most likely lameness diagnosis (e.g. fracture, claw erosion). The individual lameness diagnoses will add information to the probability distributions of the cause-category of lameness at herd level.

### **Data to the model**

The study uses results from a large number of published papers in order to quantify the conditional probability distribution between any two nodes. Where no quantitative information exists we take advantage of expert opinions. However, in some situations we have quantitative information about nodes that are not directly connected with a link in the model. For instance, it is possible to estimate the conditional probability distribution between *Weight* and *OCD* (osteocondrosis dissecans) based on information from the literature. However, what we need to

specify in the model is the conditional probability distribution between *Weight* and the cause-category: *Inherited*. Using the Markov property of a Bayesian network it is possible to move directed edges from where we have the quantitative information to directed edges that represent the causality in the model (Otto and Kristensen, 2004)

## RESULTS AND DISCUSSION

The Object-Oriented Bayesian Network model presented in this paper estimates the probability distributions for three cause-categories of lameness in finisher herds based on evidence from two classes. The biological structure of the model is shown in Figures 1–3 and a description of each node, including the node type and the states, is shown in Tables 1–2.

Using evidence from the herd class only, it will be possible to estimate the probability distributions of the three cause-categories. However, by including information from the pig class, it will be possible to obtain further knowledge and reduce the uncertainty about the cause-category of lameness in the herd. Previously, a Bayesian network model describing the infection with *Mycoplasma hyopneumoniae* in swine herds has been developed (Otto and Kristensen, 2004). In that study only herd-specific risk factors were included in order to estimate the probability distribution of the severity of infection. The differential diagnoses for lameness presented in this model are not fully complete and it is possible for pigs to have clinical signs of lameness due to other aetiologies (e.g. nerve compression and muscle rupture). However, we believe that the lameness diagnoses presented in this model are the most common in Danish finisher herds. Published research results as well as expert opinions form the basis for the qualitative and quantitative structure of the model. However, we have not taken into account the fact that the evidence used in the calculation of the conditional probability distributions, can be associated with uncertainty.

This model is the first step in developing an economic model for leg disorders in finisher herds. Hence, the probability distributions for the different cause-categories of leg disorders can successively serve as a consistent set of inputs for economic calculations of the effects of alternative control strategies against leg disorders in finisher herds. More work is needed to complete the quantitative part of the model presented in this paper.

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**Table 1.** Nodes in the herd class

Node name	Node type	Explanation	States
PenDen	Input node	The stocking density of pens	High/Low
Floor	Input node	The type of floor in pens	Solid/Partially slatted/Totally slatted
Straw	Input node	Supply of straw to pens	Deep bedding/Sparse bedding/No bedding
Herd Size	Input node	Number of pigs slaughtered annually	1-1000/1001-3001/3001-5000/5000-
Production	Input node	Type of production in the herd	Sectioned/Continues
Purchase	Input node	Number of farms that supply piglets to the herd	Zero/One/More than one
Breed	Input node	The breed of pigs	Landrace/Yorkshire/Duroc
FeedStrat	Input node	The feeding strategy	Ad libitum/Restricted
Weight	Input node	Average daily weight gain of pigs in the herd	700g/800g/900g/1000g
Environmental	Output node	Measure of the environmental causes of leg disorder in the herd	1-10
Infectious	Output node	Measure of the infectious causes of leg disorder in the herd	1-10
Inherited	Output node	Measure of the inherited causes of leg disorder in the herd	1-10

**Table 2.** Nodes in the pig class

Node name	Node type	Explanation	States
Fracture	Latent node	Fracture of the limb or claw	Yes/No
ClawErosion	Latent node	Erosion to the heel, toe or sole	Yes/No
ClawLesion	Latent node	Lesion in the white line or side wall of the claw	Yes/No
Myc	Latent node	Mycoplasma hyosynoviae	Yes/No
Strep	Latent node	Streptococcus sp.	Yes/No
Erysi	Latent node	Erysipelothrix rhusiopathiae	Yes/No
Haemo	Latent node	Haemophilus parasuis	Yes/No
OCM	Latent node	Osteochondrosis manifesta	Yes/No
OCD	Latent node	Osteochondrosis dissecans	Yes/No
Gender	Input node	Gender of the pig	Castrate/Female
MeatPercent	Input node	Lean meat percentage (increase in percent points)	0/1/2/3/4/5
TailBite	Input node	Tail bite	Yes/No
Arth_Risk	Latent node	Risk of arthritis	Yes/No
PigLame	Latent node	True state of the clinical lameness	Yes/No
Obs_Lame	Input node	Observation of clinical signs of lameness	Yes/No
Clinic1	Input node	Results of the clinical examination of ClawLesion	Yes/No
Clinic2	Input node	Results of the clinical examination of ClawErosion	Yes/No
Bac1	Input node	Results of the bacteriological examination of Mycoplasma hyosynoviae	Yes/No
Bac2	Input node	Results of the bacteriological examination of Streptococcus sp.	Yes/No
Bac3	Input node	Results of the bacteriological examination of Erysipelothrix rhusiopathiae	Yes/No
Bac4	Input node	Results of the bacteriological examination of Haemophilus parasuis	Yes/No
Path (1; 9)	Input node	Results of the pathological examination of the leg or joint. One node for each of the nine lameness diagnoses	Yes/No

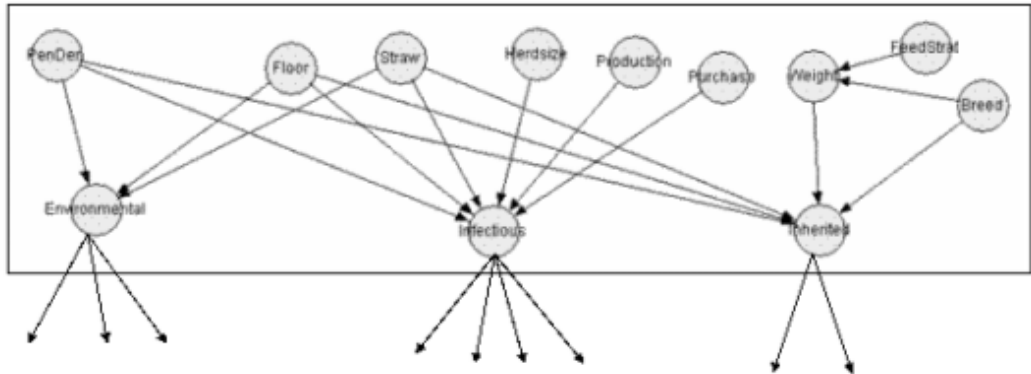


Figure 1. The herd class

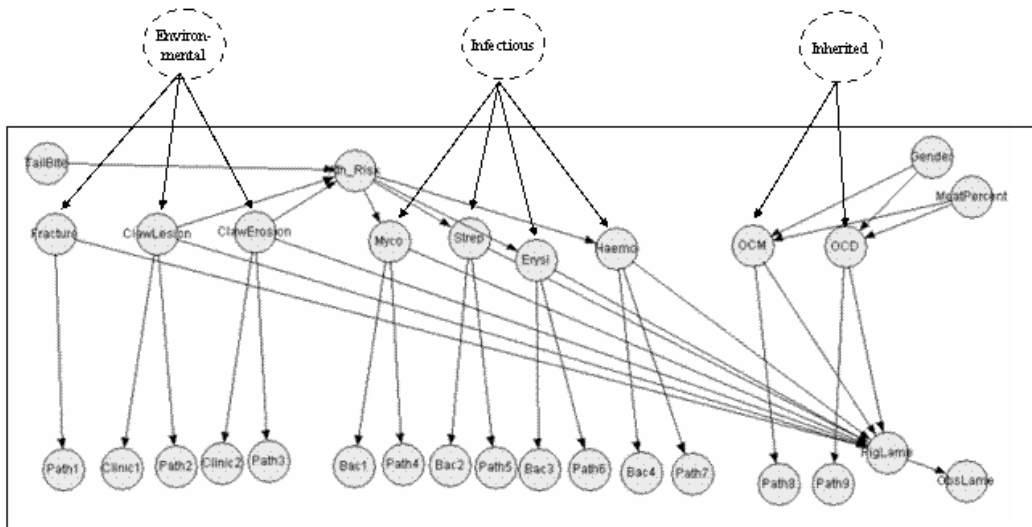
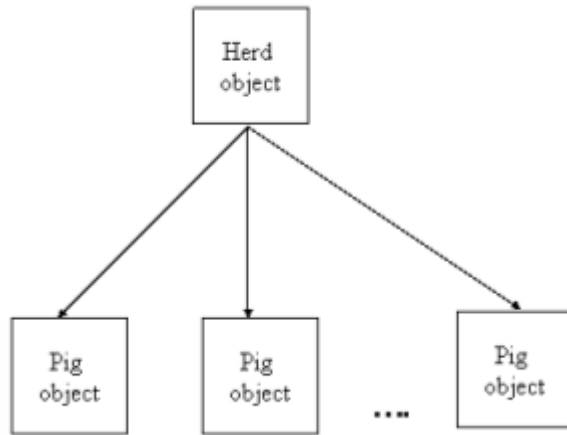


Figure 2. The pig class





**Figure 3.** The relation between objects in the herd class and the pig class

## SUBCLINICAL METABOLIC DISORDERS IN PERIPARTAL DAIRY COWS IN HUNGARY IN 2005

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### ABSTRACT

Feed intake often fails to meet the requirements of high yielding cows, especially some weeks prior to and after parturition which may induce subclinical or clinical metabolic disorders. The losses due to metabolic disorders such as decreased milk production, reproduction failures, and management related diseases e.g. lameness, mastitis etc., emergency slaughters and death of diseased animals.

In order to reveal the subclinical metabolic disorders in high yielding dairy cows in the peripartal period, metabolic profile test was carried out at 69 large-scale dairy herds with the population of approximately 35.000 Holstein-Friesian cows, aged 5–6 years on average in Hungary in 2005. In this survey groups of cows were tested as following:

Group I: dry cows, 1–10 days prior to expected parturition (n=424);

Group II: cows 1–7 days after calving (n=377);

Group III: cows 8–30 days after calving, n=546);

Group IV: cows 31–90 days after calving (n=534).

The results of the study are comparable to the figures have been obtained in the previous 10 years and reported elsewhere (Brydl et al., 1997; 1998 and Könyves et al., 2001; Brydl et al. 2003). Likewise to screening data of the previous year's high incidence of energy imbalance, aciduria (subclinical acidosis), inadequate protein supply, carotene shortage, and inappropriate sodium and potassium supply was detected. In comparison with data of the previous years no substantial change was observed with respect to occurrence rate of metabolic disorders.

**Keywords:** dairy cow, peripartal period, metabolic disorders, malnutrition

### INTRODUCTION

Remarkable genetic progress has been seen at the large-scale dairy farms in Hungary during the last decades and the milk production is more than 8000 kg in 305 days of lactation (Mészáros, 2007.). The average population number at the large-scale dairy units in Hungary is between 350 and 400 head of cows, ranging between 100 and 2000. Due to the ever-increasing genetic potential the nutritional demand of the cows are increasing and needs more sophisticated feeding strategy with improved feed quality. The upgrading genetic capacity, however, often does not meet with the nutritional intake and the welfare conditions of the cows. Feeding errors induce subclinical/clinical metabolic disorders some days/weeks prior to and especially after parturition with an increased rate of mortality, decreased production and reproduction failure.

Majority of the losses are caused by subclinical metabolic disorders. For early detection of subclinical cases metabolic profile tests (MPT) have been developed and applied all over the world since the late sixties (Payne et al. 1970, 1972 and 1973; Sommer, 1975; Brydl et al. 1987). At the Faculty of Veterinary Science Budapest a comprehensive and complex metabolic profile test (MPT) was developed and it has been used since 1985 for monitoring the metabolic status of high yielding dairy cows at many large-scale Hungarian dairy farms (Brydl et al., 1987, 1988 and 1989). For the last 10 years the data of the MPTs were summarised annually (Brydl et al., 1997 and 1998; Könyves, 2001; Jurkovich et al., 2002, Brydl et al., 2005) and attempts were made to draw conclusions of general merit and detect trends of changes.

The MPT is based not only on laboratory examinations of blood, urine, rumen fluid, pigmented hair and feed samples, but on the results of detailed farm visit as well. During the farm visits data were collected on the feed quality, the mode of feeding, the milk production, the parameters of reproduction, health status of the herd and the body condition and faces were scored in every cases.

The biological samples were taken from clinically healthy cows, assigned from various groups of cows randomly, 3–5 hours after the morning feeding. The groups differed in respect of daily milk yield, stage of lactation and gestation as well (3, 4, 5, 6, 7, 8, 17).

## MATERIALS AND METHODS

In 2005 69 large-scale dairy units were screened for the presence and prevalence of subclinical metabolic disorders. These farms housed approximately 35 000 head of Holstein-Friesian cows of 3–6 years of age. In this survey data of laboratory examinations (blood chemistry, chemical analysis of urine and hair samples) were analysed together with the actual feeding strategy (composition and quantity and quality of the daily ration), parameters of milk production and reproduction, body condition score and rate of morbidity and mortality of the farm in question.

The biological samples were taken from clinically healthy cows, selected randomly from various groups of cows with different physiological stage, 3–5 hours after the morning feeding. The groups differed in respect of daily milk yield, stage of lactation and gestation. In the present study the following groups were tested:

Group I: dry cows, 1–10 days prior to expected parturition (n=424);

Group II: cows 1–7 days after calving (n=377);

Group III: cows 8–30 days after calving, n=546);

Group IV: cows 31–90 days after calving (n=534).

The energy metabolism was monitored by the measurement of blood glucose, aceto-acetic-acid and NEFA concentration. Subclinical fat mobilisation syndrome was diagnosed by the values of NEFA and AST activity. Subclinical ketosis was revealed by the values of glucose and aceto-acetic-acid in the blood samples. Non-bypass protein supply was monitored by the determination of urea concentration in the blood and urine samples. Concentration of total carotene, calcium, inorganic phosphorus, copper, and zinc was measured in blood samples. GSH-Px activity of red blood cells was determined in order to monitor selenium supply. Acid-base metabolism was measured by the urinary pH and by the determination of NABE value in the urine samples.

## RESULTS AND DISCUSSION

Occurrence and prevalence of different forms of subclinical metabolic disorders is summarised in Table 1.

It is seen that occurrence of different form of subclinical energy imbalance was high around parturition, especially in the first week of lactation. Before calving (Group I.) and in the first week of lactation (Group II. and III) the increased fat mobilisation (high NEFA) and subclinical fat mobilisation syndrome (high NEFA and AST) dominated.

**Table 1.** Occurrence of subclinical metabolic disorders

Subclinical metabolic disorders	Sampled groups of animals			
	Occurrence of metabolic disorder ; %			
	I.	II.	III.	IV.
Increased fat mobilisation	11.1	4.5	3.1	0.9
Subclinical fat mobilisation syndrome	4.0	28.4	8.1	1.3
Subclinical ketosis	3.3	4.0	2.6	3.7
Subcl. f. m. syndrome+subcl. Ketosis	0.0	18.6	6.8	0.9
<b>Energy imbalance all</b>	<b>18,4</b>	<b>55,4</b>	<b>20,5</b>	<b>6,9</b>
<b>Aciduria</b>	59.6	63.5	45.0	29.8
– Acid load	51.3	57.4	41.5	28.9
– Imminent metabolic acidosis	8.3	6.2	3.5	0.9
Body condition score: >3,5	22.5	6.9	1.5	0.7
<3.0	5.9	18.0	48.4	68.5
Protein shortage	30.0	10.9	11.4	5.1
Protein surplus	26.4	50.4	45.1	68.0
<b>Carotene shortage</b>	63.7	84.1	76.4	57.9
Hypocalcaemia	0.2	2.7	0.5	0.2
Hypophosphataemia	6.6	24.1	15.2	12.2
Hypomagnesaemia	0.9	8.5	3.8	0.7
<b>Hyperphosphaturia</b>	11.6	22.3	23.3	17.3
Hyperphosphaturia with aciduria	10.1	18.5	11.9	7.9
Sodium shortage	34.2	38.6	12.9	7.5
Sodium surplus	16.4	19.8	46.3	55.0
N	424	377	546	534

The occurrence of fat mobilisation syndrome decreased with the progress of lactation. Highest incidence of subclinical ketosis was detected in Group II and IV. In most of the cases the subclinical ketosis concurred with fat mobilisation syndrome. It might be supposed therefore, that subclinical ketosis in majority of the cases is the consequence of increased fat mobilisation and fat mobilisation syndrome. Increased fat mobilisation among cows before calving (Group I.) could be the consequence of the high rate (22.5%) of fat (over conditioned) cows. The rate of over conditioned cows decreased, the rate of thin cows increased rapidly by the days of lactation. The other relevant causative factors of high incidence of energy imbalance could be the very high occurrence of aciduria (and its forms like acid load and imminent metabolic acidosis) in the examined period. The highest incidence of aciduria was detected in the first week of lactation (Group II.) when the energy imbalance was also at its highest rate. The prevalence and severity of

subclinical acidosis decreased by the days of lactation, but it was still high in peak lactation (Group IV.).

Since the report of Dirksen (1970) it has been known the ruminal pH has major effect on the multiplication of rumen flora and the production of volatile fatty acids which are the main sources of meeting the energy demand of ruminant. Thus optimal rumen fermentation is a key element of the energy balance of high producing dairy cows.

Our earlier data indicated strong correlation between aciduria and hyperphosphaturia. Excessive urinary excretion of phosphorus represents both financial loss to the dairy sector and environmental pollution to cope with (Könyves et al. 2001.).

Protein shortage was detected at its highest rate before calving. On the other hand protein surplus was also seen before calving. The most remarkable overfeeding with protein was in the period around peak lactation (Group IV). Number of papers of the relevant literature proved the negative effect of protein overfeeding on the energy metabolism and reproductive performance of high producing dairy cows. The protein shortage at the same time is strong limiting factor of the milk production.

Low carotene concentrations were detected in all groups of cows examined. Monodietic nutrition applied for the last ten years in most of the Hungarian large-scale dairy units, restricted opportunity of grazing and receiving freshly harvested green roughages together with the far too high price of the commercially available carotene preparations are the responsible factors of the low carotene status of cows.

In the present survey supplementation with sodium was inappropriate. The occurrence rate of hypocalcaemia was low. High incidence of hypophosphataemia and hyperphosphaturia was detected. Hypomagnesaemia was seen only occasionally.

## CONCLUSIONS

- These data indicate the necessity of improving the feeding and management practice and implement further preventive measures.
- Screening of the metabolic status of high yielding dairy farms is a good tool for detecting metabolic disturbances in time and gives opportunity to counteract.

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## **SUSTAINABILITY OF FUTURE SWEDISH DAIRY FARMING; SCENARIOS FOR ANIMAL HEALTH, ENVIRONMENT AND ECONOMY**

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### **SUMMARY**

The overall aim of this study was to describe future scenarios for dairy farm production in Sweden, and to analyse sustainability of these scenarios, using a method previously developed. Two goal visions for dairy farming were developed; Specialised Dairy Farming (SDF) with high production intensity and Mixed Dairy Farming (MDF) with increased crop rotations and large share of pasture. When quantification was performed the scenarios were evaluated concerning economics, environmental effects and animal welfare, including health. No scenario was superior in all aspects and the goal for developing sustainable dairy farm production must be guided by analysis of values.

**Keywords:** sustainable agriculture, animal health, animal welfare, dairy

### **INTRODUCTION**

The development of the dairy farming is important for environmental aspects, as well as social and economic development of the countryside in Sweden. When aiming at a sustainable dairy production it is crucial to include these issues, but animal health and welfare should also be considered.

The dairy sector is one of the most important sectors in Swedish agriculture today. The development of dairy farm production is important for environmental aspects, as well as social and economic development of the countryside. It is important to aim for a sustainable development including these issues, but also the ethical aspects of animal husbandry; we must manage our animals in an acceptable way, which means that animal health and welfare should be considered.

The overall aim of this study was to describe future scenarios for dairy farm production, and analyse the scenarios from a sustainability point of view. The specific aims were to identify conflicts between different sustainability goals for dairy farm production; to formulate future scenarios based on defined FOOD 21 sustainability goals and analyse conflicts between goals, and to evaluate the scenarios from economic, environmental and animal welfare perspectives.

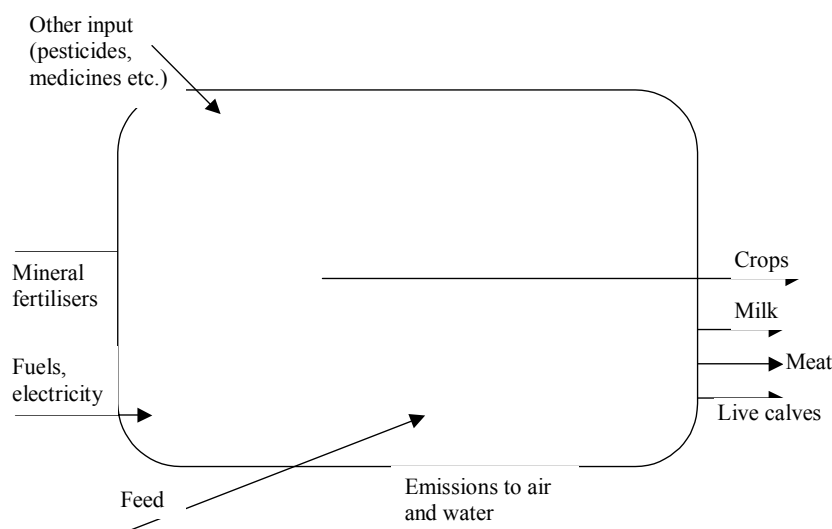
## MATERIAL AND METHODS

When scenarios were designed, it was crucial that the results reflect the basic values behind the scenarios, and that the assumptions made were clear and handled in a transparent way. In the scenario design process, several choices have to be made and the rationale for these choices must be explicit. The method used was a stepwise process where all steps were presented (Sonesson et al., 2003). The starting point was to define the parameters used to design the future scenarios. The Food 21 sustainability goals relevant for the milk farming system were mainly related to natural resources, external environmental, animal welfare and economics. A principal description of the milk production system is given in Fig 1.

Two goal visions for milk farming were developed. Each goal vision was completed with grouping of the focus parameters in respect to their relative importance for the goal vision. These groups of parameters will constitute the starting point for designing the goal vision scenarios. The two goal visions developed were;

a. Efficient production and small environmental impact per product (“High intensity”)

This goal vision was focused on efficiency, both economic and environmental. The environmental performance and resource efficiency optimised was the product oriented impact. This means that in this scenario we strived for high production per unit resource put in and per unit emission let out. The feed production was mainly based on local supply of forage feed and some grain completed with import of high quality protein feed. The production was also concentrated on milk; it was a highly specialised enterprise, which makes it possible for the staff to become specialists on dairy cows.



**Figure 1.** Principal description of the milk production system



b. Focus on animal welfare, working environment and local environmental impact (“Low intensity”)

This goal vision was focused on environmental efficiency, mainly on area level, but the production level was also taken into account. This means that the environmental impact per unit of land was minimised, but the impact per unit produced was also considered. The systems build on integration of milk and meat production based on local feed production, both forage and protein feed. The milk production was managed in a way that fits well into sustainable meat production. A second aspect of the integration was that in this goal vision was that the farm can grow more cash crops in order to optimise the crop rotation; the machinery and knowledge about crop production was a natural part of the enterprise.

Two goal vision scenarios were created from the goal vision. The goal vision “Efficient production and small environmental impact per product” resulted in a scenario we call “Specialised Dairy Farming”, since the design of the scenario based on focus scenarios, resulted in a specialised system with high intensity. The goal vision “Focus on animal welfare, working environment and local environmental impact” resulted in a scenario we call “Mixed Dairy Farming”. The focus scenarios most important for this goal vision resulted in a system where good crop rotations and large share of pasture were important.

Based on the qualitative descriptions and the design of buildings, the farming systems were quantified. The quantification was done through expertise judgement based on available statistics on agricultural production combined with general knowledge synthesising research and extension services (Statistics Sweden, 2004; Agriwise, 2005; Swedish Dairy Association, 2005). When quantification was performed the scenarios were evaluated concerning economics, environmental effects and animal welfare, including health. An environmental assessment of the two scenarios was performed, using Life Cycle Assessment. The analysis included investigation of eutrophication, global warming potential, acidification and toxicity (measured as amount of active substance of pesticides used). The use of resources for the system was quantified as energy use, land use and usage of phosphorus.

## RESULTS AND DISCUSSION

Cost of production was in scenario “Specialised Dairy Farming” 3.02 SEK/kg milk, in scenario “Mixed Dairy Farming” 4.34 SEK/kg milk, and in the present production 2.87 SEK/kg milk. The economic analysis shows that neither of the two scenarios was economically viable in the present economic context. This was due to high building costs for both scenarios and also higher labour and feed costs for scenario “Mixed Dairy Farming”. The high cost for labour in scenario “Mixed Dairy Farming” was a result of high ambitions for animal welfare and reflects the cost of a high level of animal welfare in dairy production. If the economic result had been used to refine the building design the costs would have decreased. The high cost for feed in “Mixed Dairy Farming” was somewhat complicated; the on-farm production of feed was more costly than purchased feed used in “Specialised Dairy Farming”. This was not logical since the components in the feed was largely the same, so perhaps the price of purchased feed actually does not reflect the production costs, i.e. the feed producers are not paid enough to cover their actual costs. A second explanation for the high feed costs per kg milk in scenario “Mixed Dairy Farming” was the relatively low milk production per cow.

The emissions of eutrophying emissions are especially important for agricultural production since agriculture contribute to approximately 50% of all eutrophying emissions in Sweden. The

contribution to eutrophication per litre milk was lowest for scenario “Specialised Dairy Farming”. At the same time, scenario “Mixed Dairy Farming” contributes less to eutrophication per land area used. This can be equally important since eutrophication largely is a local or regional environmental effect. Both these aspects are important and the emissions per litre milk should be an important aspect when discussing total environmental impact from dairy production. The emission per area land is important when livestock production is discussed on a regional level, in areas where the intensity is high or the receiving watershed is sensitive. The environmental assessment showed that the co-production of meat and live calves has important effect for the overall environmental impact; hence the choice of analysis method was crucial. This affects the results, mainly for the “Mixed Dairy Farming” since it produces more meat and calves. We have assumed that the alternative beef production was an extensive suckler cow production, which means that the meat and calves are saving relatively large emissions of ammonia and land use. If a more intense beef production system would have been chosen, these effects would have been lower, but energy use and emissions causing global warming potential would increase for the alternative beef production. However, the results showed the importance of including the co-products in systems analyses of this kind. (For complete report on the life cycle analysis see Sonesson, 2005)

In the scenario construction, factors that are considered to improve animal welfare were implemented in both scenarios, as the legal requirements on the animal housing in Sweden have to be met. We used areas of concern found in previous research as a guideline to investigate the potential welfare differences between the scenarios we constructed. We found that a theoretical evaluation partly would be possible, considering the scientific knowledge about how housing and management is affecting health and welfare (e.g. Bendixen et al., 1988; Bergsten, 2003; Enevoldsen & Gröhn, 1996. Hultgren, 2001, Murray et al., 1996; 1990; Singh et al. 1994).

Cows in “Specialised Dairy Farming” were found to have a higher risk of lameness as they both have and increased risk of getting heel-horn erosion and laminitis, compared to cows in the “Mixed Dairy Farming” scenario. In the “Specialised Dairy Farming” scenario, the cows were having a higher milk yield, which have been found to be associated with an increased risk of mastitis, ketosis and abomasal displacement. Furthermore, the extended access to grazing on pasture in the “Mixed Dairy Farming” scenario decreases the risk of getting mastitis, and they also have lower risk of dystochia.

Comparing the two scenarios, “Mixed Dairy Farming” probably has more positive impacts on the long term environment than “Specialised Dairy Farming”. The reason was that “Mixed Dairy Farming” involves a more varied crop rotation, which is beneficial for many biological aspects. Scenario “Mixed Dairy Farming” also uses more pasture, which can improve the biodiversity. However, the pasture was intense and hence less valuable from a biodiversity point of view. (For details see Gunnarsson et al., 2005)

The objectives of the study were met, and the process of designing the scenarios gave valuable insights and contacts within the dairy sector. The concrete way of describing the future scenarios worked well in discussions both with researchers from different fields as well as practitioners. The systematic approach; starting with defining the sustainability goals in an operational manner made a clear and logical analysis of goal conflicts possible. The transparent choice when goal conflicts appear also contributed to higher acceptance of the scenarios.

The assessments of the scenarios are complex; there are many aspects to consider simultaneously. The fact that the evaluation of scenarios was done both quantitatively and qualitatively involves difficulties with balancing the conclusions; the quantitative results often are given more weight than the qualitative ones. The results show that no scenario was superior in all

aspects. The implication of this is that the goal for developing sustainable dairy farm production must be guided by values, i.e. choices of what sustainability goals that is more important. This is an important finding in our perspective; no system is the sole solution and choices have to be made, and studies of this kind is important to see what the choices are and what the consequences of the choices are. The choice of scenarios in this study was to some extent extreme, in reality a combination of the solutions in the two scenarios were likely to be most efficient in the quest for a sustainable development. The mainly positive environmental results for scenario “Mixed Dairy Farming” must be considered as rather strong, since the assumed milk yield is rather low. However, the low milk yield results in high production costs per kg of milk.

The results from the study can function as a discussion platform, where the debate about sustainability in dairy farming can be directed towards the conflicting goals instead of towards what measures to prefer. The methodology has been applied to pig production (Stern et al., 2005), and beef production (Kumm et al., 2005).

### CONCLUSION

No scenario was superior in all aspects and the goal for developing sustainable dairy farm production must be guided by analysis of values. The economic analysis shows that neither of the two scenarios was economically viable in the present economic context. The contribution to eutrophication per litre milk was lowest for scenario “Specialised Dairy Farming”, but the “Mixed Dairy Farming” contributes less to eutrophication per land area used. Cows in “Specialised Dairy Farming” were found to have a higher risk of lameness as they both have and increased risk of getting heel-horn erosion and laminitis, compared to cows in the “Mixed Dairy Farming” scenario. No system is the sole solution and choices has to be made, and studies of this kind make it possible to survey which choices that can be made and what the consequences are.

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## EVALUATION OF BETA-HYDROXY BUTYRATE AND GLUCOSE IN SUBCLINICAL KETOSIS IN INDUSTRIAL HERDS OF HOLSTEIN COWS

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### SUMMARY

Subclinical Ketosis (SCK) is one of the most prevalent metabolically disorders found commonly in dairy farms worldwide which is caused by lack of balance in diet and energetic deficiency in animals. The objective of this study was to study of BHB and glucose levels in healthy Holstein cows and cows with SCK and to determine of the prevalence of the disease, using BHB level in blood serum as the gold standard. In this study 7 dairy farms were chosen in Shahriar, (Tehran province, Iran). Samples were taken from 100 cows at two periods: 1) last week of pregnancy (dry period), 2) 1, 2, 4 and 8 weeks after parturition. Serum samples were harvested and BHB levels were measured, using RANBUT kits (Randox, England) and glucose levels was measured by commercial available kits (Ziest Chimi, Iran) using spectrophotometer. In this study, the prevalence of SCK, using 1.2, 1.4 and 1.7 mmol/L BHB as the cut-off point were calculated as 18% and 14% and 4%, respectively. In this study the mean levels of BHB, in two-month-post parturition group was higher than the cows at their last week of pregnancy. Mean glucose levels in cows at two months after parturition and also in cows with SCK were lower than in cows at their last week of pregnancy and healthy cows at two months after parturition.

There was a significant correlation coefficient ( $r = -0.27$ ,  $P < 0.05$ ) between BHB and glucose levels in cows at their last week of pregnancy. Correlation coefficient analysis also showed a relationship between BHB concentration and glucose levels ( $r = -0.64$ ,  $P < 0.05$ ) in cows at their second months after pregnancy. The correlation between BHB and glucose levels in cows affected by SCK was not significant ( $P > 0.05$ ). There was a relationship between BHB and glucose levels ( $r = -0.53$ ,  $P < 0.05$ ) in the healthy cows at second month after pregnancy.

**Keywords:** subclinical ketosis, glucose, BHB, cattle

### INTRODUCTION

Subclinical ketosis is the accumulation of large quantities of ketone bodies in blood and tissues. Ketone bodies include  $\beta$ - Hydroxy butyric acid, acetoacetic acid and acetone. The maintenance of adequate concentrations of glucose in blood is critical to the regulation of energy metabolism. In ruminants carbohydrates are fermented in the rumen to fatty acids principally acetate, propionate and butyrate. Propionate and amino acids are the major precursors for gluconeogenesis with glycerol and lactate of lesser importance (1, 5, 18).

The initial event in the pathogenesis of ketosis is negative energy balance and the accompanying mobilization of non esterified fatty acids from adipose tissue. Negative energy balance is prevalent in dairy cows during the first 2 to 8 weeks of lactation since feed intake doesn't keep pace with the rapid increase in energy demands for milk production. Ketosis may be clinical or subclinical and effected milk production and reduced reproduction (6, 7,8).

The economic impact of ketosis is derived from treatment costs, reduced milk production and reduced fertility. The disease is seldom fatal, so death loss isn't an important economic factor (9, 10).

Clinical ketosis is frequently associated with concurrent disease both infectious and metabolic. In many cases, ketosis occur secondary to another disease. In other instances, ketosis may be the initial disease (18, 19).

Clinical ketosis cause gastrointestinal and nervous sings. SCK often is without clinical sings and cause drop in milk production, reduced fertility and partial anorexia that result in less body condition. Diagnosis of SCK is important for prevention of economic losses (11, 12, and 14). The objective of this study was to study the BHB and glucose levels in healthy Holstein cows and cows with SCK and to determine the prevalence of SCK, using BHB levels in blood serum as the gold standard.

## MATERIAL AND METHODS

In this survey 7 dairy farms were chosen in Shahriar, Tehran province, Iran. Samples were taken from 100 cows at two periods: 1) last week of pregnancy (dry period), and 2) 1, 2, 4 and 8 weeks after parturition. Blood samples were taken from jugular veins and serum was harvested by 3000 rpm centrifuge, for 10 min. BHB levels were measured using RANBUT kits (Randox. England) and glucose levels were measured by commercial kits (Ziest Chimi, Iran) using spectrophotometer (Biowave F 2100) (20,21).

## STATISTICAL ANALYSIS

Paired student's t-Test was used to evaluate the differences between groups. Simple linear correlation was used to find the relationships between the variables, using SPSS 10 for Windows.

## RESULTS

In this study, the prevalence of SCK using 1.2, 1.4, and 1.7 mmol/L BHB, as the cut-off points for detection of SCK, calculated as 18% and 14% and 4%, respectively at two months after parturition. The results are shown in table 1. Results of the biochemical blood tests are shown in Table 2.

**Table 1.** The prevalence of subclinical ketosis at two months after parturition

Prevalence of subclinical ketosis prevalence	BHB (1.2 mmol/L)	BHB (1.4mmol/L)	BHB (1.7 mmol/L)
	18%	14%	4%

**Table 2.** BHB and glucose mean levels ( $\pm$  SD) in cows after and before parturition and in cows with sub clinical ketosis and healthy

Time	1) Healthy cows before parturition	2) Healthy Cows after parturition	3) Sub clinical ketosis cows	4) Healthy cows
BHB mmol/L	0.48 $\pm$ 0.22 <sup>2</sup>	0/61 $\pm$ 0/52 <sup>1</sup>	1/67 $\pm$ 0/12 <sup>4</sup>	0/44 $\pm$ 0/31 <sup>3</sup>
Glucose mg/L	49/78 $\pm$ 11/28 <sup>2</sup>	42/78 $\pm$ 17/34 <sup>1</sup>	23/14 $\pm$ 4/31 <sup>4</sup>	45/98 $\pm$ 16/54 <sup>3</sup>

\* (1–4) Means within a row with common superscript differ significantly ( $P < 0.05$ )

## DISCUSSION

SCK (also called acetoanaemia) occurs in higher yielding cows in early lactation. Acetone is produced by the liver and released into the blood where it acts as an intoxicant to the cow. The disease is caused by an inadequate intake of “starchy” foods in a cow, which is already mobilizing body fat. SCK is a disease of dairy cattle and is prevalent in most countries where intensive farming is practiced. The occurrence of the disease is very much dependent upon management and nutrition. One of the energy metabolism parameters monitored in this study was blood glucose concentration. Statistically significant differences between the two groups of dairy cows (before and after parturition) and between healthy and SCK cows were found ( $P < 0.05$ ).

The mean level of glucose in cows at two months after parturition and also in cows with SCK was lower than the cows in their last week of pregnancy and healthy cows in two month after parturition. Decrease in blood glucose concentrations reported in response to fat supplementation in the first stage of lactation in dairy cows. Our results are in accordance with the results of other studies (5, 13, 15, 16, 17, and 23). Glucose is a substance that plays a fundamental role in all animals. In the last weeks of fetal development, the fetus uses around 46% of maternal glucose taken up by the uterus. Additionally, a cow producing 30 kg of milk per day uses at least 2 kg of blood glucose to synthesize lactose for milk. The end of pregnancy and the beginning of lactation, therefore, represent a time when there is a massive increase in need for glucose. This poses an enormous challenge for the liver that has to synthesize all of this glucose from propionate and amino acids as well as a challenge for other tissues and organs that have to adapt to a reduction of glucose availability. Glucose is an equally important energy source for the ovary and the reduced glucose availability in the beginning of lactation can negatively impact the reestablishment of ovarian activity after calving (2, 3).

Another parameter of energy metabolism monitored was the blood concentration of BHB. Compared with glucose, BHB is a more sensitive indicator of energy metabolism disruptions, and its concentrations are increased by lipid mobilization. In our study, BHB concentrations in the SCK group at week 8 post partum were higher than in healthy groups and BHB concentrations in

cows after parturition was higher than the cows before parturition ( $P < 0.05$ ). Our results were similar with other studies (4, 5, 6, 13, 16, 17, 22, and 23).

There was a significant relationship ( $r = -0.27$ ,  $P < 0.05$ ) between BHB and glucose levels at last week of pregnancy. There was also a significant relationship ( $r = -0.64$ ,  $P < 0.05$ ) between BHB and glucose levels in their second month after pregnancy.

No significant relationship was found between BHB and glucose levels in cows affected by SCK ( $P > 0.05$ ).

Correlation coefficient analysis in the healthy cows at second month after pregnancy showed a relationship between BHB and glucose levels ( $r = -0.53$ ,  $P < 0.05$ ).

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## POSTER PRESENTATIONS

### COMPARATIVE HYGIENE ASSESSMENT OF TECHNOLOGIES FOR ORGANIC MANURE UTILIZATION WITH HIGH CONTENT OF DRY MATTER 2. TOXIC CHEMICAL ELEMENTS

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#### SUMMARY

Comparative research is carried out with 3 technologies for utilization of organic manure with high dry matter content: manure from no litter breeding of animals and immovable litter from broilers. The first technology is studied at a laboratory for compost. The second technology is methane fermentation, which is conducted with laboratory bioreactor with microprocessor control and the third technology is a combination of the two.

The comparative analysis showed that the highest losses of nitrogen, calcium and phosphorus are in composting, and the lowest in the third technology. For all 3 technologies the content of toxic microelements is under the MRLs registered in regulation 22/2001 for animal biological production.

**Keywords:** methane fermentation, compost, manure

#### INTRODUCTION

Litter from broiler production is a source used for increasing soil fertility. Baykov /2003/ proves that there are possibilities for decontamination of litter by temperature treatment and its following utilization as supplement in ruminants' ratio or for increasing soil fertility, including organic production. Methane fermentation technology for litter treatment is very prospective. Some of our research shows that manure obtained from 150 000 laying hens per year is a source of energy equal to the energy obtained from 91,25 t of petroleum /Baykov & Tyrawska, 1991/. During the last few years greater attention is paid to compost as a source of biogenic chemical elements in optimal ratios for plants /Baykov et al., 2003/. Among the admissible soil fertility products, in Ordinance №22/2001 of the Ministry of Agriculture and Forests for organic production of plants, is listed the product obtained after methane fermentation of manure which is known as compost in the United States and as bioslime in Europe.

The aim of the present research is to make an ecological assessment of compost according to the requirements of Ordinance №22/2001 for the MRL/Maximum Residue Level/ values of toxic elements and according to the Norms of the Canadian Ministry of Agriculture/2002/ and to

compare the obtained results to the results obtained in composting and those from the new technology developed by our team.

## MATERIAL AND METHODS

Research is carried out with litter from broiler production of four-line hybrid broilers for 45 days. The obtained litter is treated with water – the dry matter in the suspension is 7%. The suspension is then placed in a microprocessor controlled laboratory bioreactor, where a temperature of 33°C is maintained and the fermentation time is 15 days as determined by the model of Chen & Hashimoto /described in details by Baykov & Tyravska, 1991/. The obtained compost is analyzed for dry weight and for content of toxic chemical elements by applying the methods described by Jorchem /1993/ with AAS “Perkin-Elmer-4100”. Parallel to the results in table 1 are listed the results of the analysis of toxic elements in litter after composting as well as results from the developed technology by our team for litter treatment together with calcium oxide and water in the ratio: 75% litter, 20% Ca(OH)<sub>2</sub> and 5% water. Table 1 indicates the results of 12 experiments with methane fermentation of manure from laying hens.

**Table 1.** Content of toxic elements in products after litter treatment

Element Group	Ca mg/kg	Mg mg/kg	Pb mg/kg	Cd mg/kg	Hg mg/kg	As mg/kg	Cu mg/kg	Cr mg/kg	Zn mg/kg
<b>1<sup>st</sup> group</b> x ± SD	48519,99 ± 11312,00	9399,24 ± 900,43	1,49 ± 0,6	0,27 ± 0,01	0,15 ± 0,06	0,69 ± 0,33	96,7 ± 8,86	28,5 ± 44,1	407,1 ± 38,6
<b>2<sup>nd</sup> group</b> x ± SD	66659,01 ± 19342,75	11502,13 ± 1427,33	2,05 ± 1,67	0,62 ± 0,25	0,22 ± 0,05	1,02 ± 0,30	213,8 ± 21,4	49,3 ± 72,4	477,7 ± 159,1
<b>3<sup>rd</sup> group</b> x ± SD	35439,296± 7320,08	8616,92 ± 1007,65	0,91 ± 0,31	0,36 ± 0,16	0,036 ± 0,01	0,23 ± 0,05	125,5 ± 20,8	146,3 ± 224,4	587,8 ± 187,8
<b>4<sup>th</sup> group</b> x ± SD	25173,996± 3913,15	4466,44 ± 392,70	0,42 ± 0,09	0,08 ± 0,03	0,022± 0,002	0,16 ± 0,07	54,4 ± 3,6	10,4 ± 5,7	195,5 ± 13,8

Key: 1<sup>st</sup> group – untreated litter, 2<sup>nd</sup> group – compost obtained after methane fermentation, 3<sup>rd</sup> group – compost, 4<sup>th</sup> group – mixture of litter

## RESULTS AND DISCUSSION

The results of the experiments are presented in Table 1. Our previous research /Baykov and Chukanov, 2004/indicates that diluting the substrate to 7% is rational with a view to optimizing methane fermentation. 49.2% degradation of organic substance is reached, i.e. a reserve of biogenic chemical elements in accessible/inorganic/ form and in organic compounds is obtained. This characteristic makes the long impact on soil fertility possible.

This research allows us to make an ecological assessment of the content of toxic chemical elements in compost. For 7 of these elements there are normative documents for MRL values in Ordinance №22/2001. The quantities of the analyzed chemical elements for the three technologies are below the MRLs for toxicity. Requirements do not exist in Ordinance №22/2001 for some toxic chemical elements which are important for soil fertility and for the proper function of soil

biocenoses. According to Stancheva/2000/ the phytotoxicity of toxic elements is expressed in the following order: Cu>Ni>Co>Mn>Zn. The acquired results should be interpreted according to the normative documents of other countries, too. We point out the requirements in the normative documents of the Ministry of Agriculture in Canada, which are analogical to those in the United States. It is evident that the requirements for the content of toxic elements in compost are lower in these two countries in comparison with Bulgarian requirements. So if we produce organic plant production, on the basis of these requirements, the quantities of the 10 toxic elements in compost are considerably lower than the MRL values.

The research that was carried out indicates that, in the conditions of the used technology, which parameters are determined by mathematical modeling preceded by laboratory experiments, a certain level of degradation is reached and all small molecules of organic compounds, which characterize the odor of manure, are mineralized. According to other research of ours it is determined that, at the same fermentation regime, all pathogenic microorganisms and eggs of helminthes are exterminated. The consistence of the manure also changes and this makes it possible to disperse it with machines used for fertilizer dispersion. Our results and the experiments of other countries /Al Seadi & Bo Holm – Nielsen, 2002/ are in the same direction: utilization of manure for biogas production solves the energy problems, but in the last few years the qualities of compost are of equal importance as well. According to our research, carried out for the first time in Bulgaria, it is determined that the compost, obtained from the methane fermentation of the laying hens' manure, contains lower quantities of toxic chemical elements than the MRL quantities mentioned in Ordinance №22/2001, that's why this compost can be used in organic plant production.

This study shows that in the two alternative technologies (composting and Ca(OH)<sub>2</sub> treatment) we obtain product suitable for increasing soil fertility that meets the toxic element requirements for organic production.

## CONCLUSIONS

1. Methane fermentation of laying hens' manure, with 7% dry matter content, allows a high mineralization level to be reached, where the content of dry matter decreases to 4.4%.
2. Compost, obtained by methane fermentation of manure, contains lower levels of 7 basic toxic elements (Pb, Cd, Hg, As, Cu, Cr, and Zn) than the MRLs for organic plant production according to Ordinance №22/2001 of the Ministry of Agriculture and Forests.

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## ASSESSMENT OF DISTRIBUTION OF THE LEAD AND CADMIUM IN THE RABBIT'S ORGANISM THROUGH KLARK OF DISTRIBUTION (KD)

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### SUMMARY

Investigation was conducted with 60 rabbits of New Zealand's breed divided in 4 groups in 60 days age. The animal ration includes different amount of lead and cadmium: group 1-the amount of tow toxic elements is under the MRL. The amount of tow toxic elements in group 2, 3 and 4 is 10, 100, 1000 times bigger than MRL respectively (Pb=0.2, Cd=0.1 mg/kg).

The dynamics of distribution was studied using criteria: Klark of distribution (Kd), which is the ratio between the concentration of chemical element in mg/kg product and medium Klark. Differences of the distribution of the lead and cadmium in organs and tissues of the rabbits were established.

**Keyword:** lead, cadmium, Klark of distribution, rabbits

### INTRODUCTION

Understanding the global pattern of contamination in biota is useful in evaluating the health of individual species, population and communities, and in assessing potential risks to humans. Heavy metals can enter the food chain from natural and anthropogenic sources, and once in the body, are distributed among tissues or excreted (Burger et al 2002, Mochizuki et al 2002). With each step of the food chain, concentrations increase, resulting in bioamplification, with toxicodynamics differing among spaces. Top-level carnivores or omnivores are often used as bioindicators because they usually have much higher levels of contaminants than those that are lower on the food chain (Baykov et al 2003, Chan et al 2004).

The amount of lead and cadmium which distributes in the organs and tissues of the animals depends on the interval of exposure, the quantity ingested; the production and reproduction phase of the animals, as well as their age and breed (Baykov et al 2003). Elements toxicity upon the biological systems of animals is affected by the route and form of ingestion as well as by the interaction between essential and toxic elements. Some metals are essential for life, others have unknown biologic function. Those causing poisonings are the ones, which accumulate in the body through the food chain, water and air (Gotal et al 2002 Wayland et al 2001).

The dates in literatures have not criteria for assessment bioaccumulation or distribution of lead and cadmium in the organs and tissues of the animals

The objective of our study was to determine cadmium and lead concentration in selected organs and tissues of the rabbits, and to estimate of the distribution dynamics of the lead and cadmium in the organism through criteria (suggested by us) that is Klark of distribution (Kd).

## MATERIALS AND METHODS

Investigation was conducted with 60 rabbits of New Zealand's breed divided in 4 groups in 60 days age. The four groups are equalized by origin, sex and biomass. The animal ration includes different amount of lead and cadmium:

1. Group I-the amount of the tow toxic elements is under the MRL (Maximum Residues Limit).
2. Group II-the amount of the tow toxic elements is 10 times bigger than MRL.
3. Group III-the amount of the tow toxic elements is 100 times bigger than MRL.
4. Group IV-the amount of the tow toxic elements is 1000 times bigger than MRL

Before analysis the samples were kept at  $-18^{\circ}\text{C}$ . in the laboratory the samples were weighted (2 g) and ashed with diluted nitric acid p.a. ( $\text{HNO}_3:\text{H}_2\text{O} = 2:1$ ) at  $130^{\circ}\text{C}$  for 2 h. undissolved particles were filtered off and the solution diluted to 25 ml. the digested samples were analyzed for the presence of cadmium and lead by using an atomic absorption spectrophotometer (AAS). The sensitivity for cadmium and lead was 0.0001 and 0.0005 mg/l respectively.

The dynamics of distribution was studied using criteria: Klark of distribution (Kd), which is the ratio between the concentration of chemical element in mg/kg product and medium concentration of the same chemical element in the organism.

For statistical analysis Origin® 7.0 SR0, V 7.0220 (B220) and Excel were used. The following variations of the analysis of variance (ANOVA) test were used for analysis of data. The criterion for significance was  $P < 0.05$ .

## RESULTS AND DISCUSSION

Data is presented in Table 1 and 2 for the content of the lead and cadmium in the organs and tissues of the rabbits. The concentration of lead in the lever, kidney, heart and lungs of the rabbits in the control group are 0.097, 0.100, 0.033 and 0.043 mg/kg wet tissue respectively, as well as in the experimental groups with 10, 100 and 1000 fold bigger MRL the medium content of the lead gradually increase and reach in the group with 1000 fold bigger MRL for the same organs and tissues to 1.027, 2.357, 0.055 and 0.138 mg/kg wet tissue respectively (table 1). The higher concentration of the lead is in the kidney for all groups ( $P < 0.05$ ), but the lower concentration is in the muscles ( $P > 0.05$ ). The concentration of Cd in the all organs and tissues differed significantly ( $P < 0.05$ ). The higher content of Cd in the kidney was established (table 2) (Massanyi et al 2003, Chan et al 2004).

The Cd concentration in the lever of rabbits increase significantly ( $P < 0.05$ ) form 0.772 for control group to 12.487 mg/kg wet weight for IV-group (table 2). The mean content of the cadmium in the range between 0.023 in the control group to 1.777 mg/kg wet weight in the IV group ( $P < 0.5$ ). The higher content of the Cd in the lungs is 0.818 mg/kg in the IV group. The concentration of Cd in the muscles exceed MRL (0.05) in the experimental groups, which are with 100 and 1000 fold bigger MRL while in the II group is around MRL (0.046). The lower concentration of the Cd is in the bones, where is 0.004, 0.0137, 0.015 and 0.184 mg/kg wet weight respectively for four groups. Same results were established in the rabbits, roe deer and mousse (Massanyi et al 2003, Gotal et al 2002, Exon et al 1979).

The analysis of the data in the literatures show only establishment of the contents of the lead and cadmium in the vary animal's organs and tissues (Massanyi et al 2000, 2003, Exson et al

1979, Chan et al 2004, Medvedev1999), but no information for the real distribution dynamics of lead and cadmium in the animal's organism, for that reason we suggest application new criteria Klark of distribution, which is show distribution dynamics of the lead and cadmium, which enter in the organism through fodder and water in the vary organs and tissues of animals or rabbits, which are object of the our study.

Data for Klark of distribution of lead and cadmium are presented in table 3 and 4 respectively. Medium Klark of the lead in the four groups increase with increase it level in the fodder. The analysis of results in the table 3 show tows directions, decrease and increase of the values of Kd with increase of the dose. Kd in the lever, kidney and bones increase gradually (figure 1). The Kd of lead in lever of rabbits for control group is 2.37 and increase to 2.67, 3.48 and 4.17 respectively for experimental groups. Data for kidney is 2.44, 3.94, 3.87 and 9.85 respectively for I, II, III and IV. For bones-5.71, 7.06, 7.96 and 8.30. The tendency in three organs and tissues is equally (figure 1).

The Klark of distribution of cadmium in the heart, lungs and muscles decrease according with increase of the dose in fodder proportionally (table 3).

Medium Klark of the cadmium in the organs and tissues increase according with increase of the dose in the fodder. Data for distribution of the cadmium is presented in table 4. The values of Kd of cadmium in lever begin with 11.70 for control group and decrease to 8.88 and 7.46 in II and III groups respectively, afterwards increase to 7.61 in IV group comparison with III group (figure 2a). The Kd of Cd in the muscles decrease proportionally for all groups (table 4 and figure 2b). In remaining organs and tissues the values of the Kd are increased with several characteristics for every organ and tissue.

The Kd of Cd in the kidney of animals from control group is 21.11 and increase to 25.41, 33.59 and 41.32 respectively for II, III and IV experimental groups (figure 2a). For the heart, Kd in the control group is 0.35 and increase to 0.55 in II group, but this value decrease to 0.45 in III group and increase to 1.08 in IV group. The Kd of Cd in lungs increase according increase the dose of the cadmium in the fodder with the exception of Kd in II group that decrease cooperation with the control group. For the muscles the values of Kd of Cd decrease according with increase the dose proportionally (figure 2b).

## CONCLUSIONS

1. The concentration of lead decrease in the lungs, heart and muscles, and increase in the lever, kidney and bones according with increase of the dose in fodder. The concentration of Cd decrease in the lever and muscles, and increase in remaining organs and tissues.
2. Criteria "Klark of distribution" allow to assessment of the bioaccumulation and distribution of the lead and cadmium in different organ and tissue.
3. The differences of steppe of the distribution of lead and cadmium give new explanations for bioaccumulation of tow toxic elements, respectively for importance in its accumulation in the studded organs and tissues, which connected with the mechanism of effect on kidney and bones (Itai Itai disease) and unfavorably effect on the function of heart.



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**Table 1.** Lead content in organs and tissues of rabbits (mg/kg wet weight)

	I	II	III	IV
<b>Liver</b>	0.097 ± 0.03	0.216± 0.03*	0.397± 0.09*	1.027 ± 0.12*
<b>Kidney</b>	0.100± 0.01	0.319± 0.08*	0.441± 0.12*	2.357± 1.16*
<b>Heart</b>	0.023 ± 0,01	0.033±0,002	0.036 ± 0,01	0.055 ± 0,02
<b>Lungs</b>	0.043 ± 0,01	0.069± 0,03	0.081± 0,04	0.138 ± 0,06*
<b>Muscle</b>	0.022±0,005	0.030± 0,002	0.031±0,003	0.033 ± 0,011
<b>Bones</b>	0.234 ± 0,06	0.572± 0.18*	0.908 ± 0.27*	2.043 ± 0,80*
<b>Fodder</b>	0.58	1.08	10.80	74.74

\* significantly comparison with control group (P < 0.05)

**Table 2.** Cadmium content in organs and tissues of rabbits (mg/kg wet weight)

	<b>I</b>	<b>II</b>	<b>III</b>	<b>IV</b>
<b>Liver</b>	0.772 ±0.16	1.430±0.29*	1.933 ±0.07*	12.487±0.65*
<b>Kidney</b>	1.393 ±0.36	4.193±1.70*	8.700 ±0.72*	67.77±10.66*
<b>Heart</b>	0.023 ±0.01	0.091±0.02*	0.117±0.04*	1.777±0.38*
<b>Lungs</b>	0.023 ±0.01	0.049 ±0.01*	0.119±0.03*	0.818±0.10*
<b>Muscle</b>	0.019 ±0.01	0.046±0.02	0.051±0.01*	0.248±0.09*
<b>Bones</b>	0.004 ±0.00	0.014 ±0.002*	0.015 ±0.00*	0.184 ±0.04*
<b>Fodder</b>	0.22	1.75	5.32	45.14

\* significantly comparison with control group (P < 0.05)

**Table 3.** Klark of distribution of lead in rabbit's organs and tissues (Kd)

	<b>I</b>	<b>II</b>	<b>III</b>	<b>IV</b>
<b>Liver</b>	2.37	2.67	3.48	4.17
<b>Kidney</b>	2.44	3.94	3.87	9.58
<b>Heart</b>	0.59	0.41	0.32	0.22
<b>Lungs</b>	1.05	0.85	0.71	0.56
<b>Muscle</b>	0.54	0.37	0.27	0.13
<b>Bones</b>	5.71	7.06	7.96	8.30

**Table 4.** Klark of distribution of cadmium in rabbit's organs and tissues (Kd)

	<b>I</b>	<b>II</b>	<b>III</b>	<b>IV</b>
<b>Liver</b>	11.70	8.88	7.46	7.61
<b>Kidney</b>	21.11	25.41	33.59	41.32
<b>Heart</b>	0.35	0.55	0.45	1.08
<b>Lungs</b>	0.35	0.30	0.46	0.50
<b>Muscle</b>	0.29	0.28	0.20	0.15
<b>Bones</b>	0.06	0.08	0.06	0.11

## PRION PROTEIN GENE (PRP) POLYMORPHISMS IN SCRAPIE AFFECTED INDIGENOUS GREEK GOATS (*Capra prisca*)

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### ABSTRACT

Scrapie is a neurodegenerative disease, belonging to transmissible spongiform encephalopathies group. In goats, unlike to sheep, no strong association between certain prion protein (PrP) polymorphisms and scrapie has been determined. In our study, 33 affected goats from scrapie outbreaks in Greece were genotypically analyzed. The main detected polymorphisms are referred to codons 17, 22, 28, 30, 127, 143, 154 and 196. No polymorphisms have been found in codons 142, 154 and 236. The predominant genotype was MM<sub>17</sub>GG<sub>22</sub>PP<sub>28</sub>PP<sub>30</sub>GG<sub>127</sub>II<sub>142</sub>HH<sub>143</sub>RR<sub>154</sub>TT<sub>196</sub>II<sub>236</sub> (15/33 goats). The usual five octapeptide repeat in codons 54–102 revealed either polymorphisms or partial deletion.

**Keywords:** scrapie, goats, polymorphisms

### INTRODUCTION

Scrapie is a neurodegenerative disease of sheep and goats that belongs to the transmissible spongiform encephalopathies (TSEs) group, linked to genetic background. In goats, unlike sheep, no strong association between certain prion protein (PrP) polymorphisms and scrapie has been determined. This absence of polymorphisms associated to the sensitivity/resistance renders difficult the establishment of breeding programs for scrapie resistance in goats, compared to sheep (European Union Decision 2003/100/EC).

The polymorphisms (amino acid substitutions) described so far in caprine PrP are V21A, L23P, G37V, G49S, W102G, T110N, T110P, G127S, I142M, H143R, N146S, R151H, R154H, P168Q, R211Q, I218L, Q220H, Q222K and S240P (Goldmann et al., 1996, 1998, 2004; Wopfner et al., 1999; Billinis et al., 2002; Agrimi et al., 2003; Zhang et al., 2004; Kurosaki et al., 2005; Acutis et al., 2006; Papisavva-Stylianou et al., 2006). The W102G polymorphism has been found only in combination with a PrP variant containing three instead of the usual five octapeptide repeats (Goldmann et al., 1998). Silent mutations have been also described at codons 42 (a→g), 107 (g→a), 138 (c→t), 207 (g→a) and 231 (a→c) (Goldmann et al., 1996; Billinis et al., 2002; Zhang et al., 2004).

The aim of the present study was to determine and describe the PrP genotype of scrapie affected indigenous Greek goats (*Capra prisca*), in order to further proceed to genetic association studies.

## MATERIALS AND METHODS

In our study, 33 scrapie affected goats (National Surveillance TSEs Programme 2003–2006, Greek Ministry of Rural Development and Food) were genotypically analyzed. Genomic DNA was isolated from frozen brain tissues by using manual PUREGENE™, DNA Purification system (Cell & Tissue Kit, D-5500A, GENTRA systems). DNA products were evaluated for their quantity and quality, using electrophoresis (Sambrook et al., 1989). PCR amplification of the entire Open Reading Frame (ORF) of PrP gene was performed in two steps using two subsequent PCR reactions: first PCR reaction uses the universal primers M13F and M13R, that anneal about 24bp further of the extreme 5' and 3' regions of the PrP-coding sequence, respectively and second “nested” PCR reaction using primers G1-G2 (Billinis et al., 2002) to specifically amplify the 768bp ORF fragment (Table 1).

Amplification reactions were performed in a PTC-200 Cyclor and products were visualized by staining with ethidium bromide after the electrophoresis of an 8 µl reaction mixture on 1% agarose gels. PrP polymorphisms were detected twice by DNA sequencing on both strands of the M13F-M13R and G1G2 PCR products. In the present study, genotypes are described by the single letter amino acid code.

## RESULTS

The main polymorphisms detected are related to codons 17, 22, 28, 30, 127, 142, 143, 154, 196 and 236 (Table 2). The predominant genotype was MM<sub>17</sub>GG<sub>22</sub>PP<sub>28</sub>PP<sub>30</sub>GG<sub>127</sub>II<sub>142</sub>HH<sub>143</sub>RR<sub>154</sub>TT<sub>196</sub>II<sub>236</sub> (15/33 goats or 45.45%), which is considered as the “wild type” (reference to *C. hircus* sequence of Barbieri and Capucci, BLAST GenBank, EF139167, GI: 119489905). Sequencing results revealed amino acid alterations before codon 37, and specifically a dimorphism in codon 17 (L/R17M), a polymorphism in codon 22 (C22G) and in codon 30 (T30P).

In 8 affected goats, the usual five octapeptide repeat in codons 54–102 (Goldmann et al., 1998) revealed either polymorphisms or was partly deleted (1 goat). The W102G polymorphism has been found in two cases without the combined presence of three instead of the usual five octapeptide repeats (Goldmann et al., 1998).

For 142, 154 and 236 codon polymorphisms, which are widely correlated to natural scrapie, the following were detected:

- in codon 142, the only detected substitution was I (Isoleucine)
- in codon 154, R (Arginine) was predominant, except for two cases where L (Leucine) was present and
- for codon 236, 54.55% of the animals did not possess the “protective” amino acid I (Isoleucine). P (Proline) was present in 12.12% of the goats, in combination with a new polymorphism concerning M (Methionine) in 6.06% of the goats.

## CONCLUSIONS

The predominant genotype MM<sub>17</sub>GG<sub>22</sub>PP<sub>28</sub>PP<sub>30</sub>GG<sub>127</sub>II<sub>142</sub>HH<sub>143</sub>RR<sub>154</sub>TT<sub>196</sub>II<sub>236</sub> has no similarity to previous reported variants in *Capra prisca* (Billinis et al., 2002) except for the observation that almost half of the animals tested (45.45%) carried the genotype HH<sub>143</sub>RR<sub>154</sub>. Previous findings relating the genotype GG<sub>127</sub>II<sub>142</sub>HH<sub>143</sub> (Acutis et al., 2006) to potential protection to scrapie, was not confirmed here.

No polymorphisms were found in codons 146 or 151 (Papasavva-Stylianou et al., 2006) and usual five-octapeptide repeat was present in 25 scrapie affected goats. Previously, unreported polymorphisms were detected in codons 17, 22 and 30, but more research needs to be carried out in the healthy *Capra prisca* population to detect genetic association(s) to the disease. Further data also need to be collected for verification of the “protective” effect of polymorphism in codon 236.

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**Table 1.** PCR conditons.

Target gene	Target codons	Primers (5'→3')	Fragment size (bp)	Master mix (50 µl / reaction)	Cycler conditons
<i>PrP gene</i> (Open Reading Frame)	-9 to 256+8	<b>M13 F:</b> – CAG GAA ACA GCT ATG ACC GAT AAT GAA AAC AGC AAG GTT GCC <b>M13 R:</b> – TGT AAA ACG ACG GCC AGT CCC TCT TTA TTT TGC AGA GAA GTC	779	0.5–1 µg genomic DNA, 0.8 mM dNTPs, 1.5 mM MgCl <sub>2</sub> , 3.5 units Taq-polymerase (Invitrogen), 20 pmol of each primer.	94°C/4'- 94°C/30"x40 <b>59°C/1'x40</b> 72°C/30"x40 -72°C / 5'
	1 to 256	<b>G1:</b> – ATG GTG AAA AGC CAC ATA GGC AGT– <b>G2:</b> – CTA TCC TAC TAT GAG AAA AAT GAG–	768	1µl M13 PCR product, 0.8 mM dNTPs, 1.5 mM MgCl <sub>2</sub> , 3.5 units Taq-polymerase (Invitrogen), 20 pmol of each primer.	94°C/4'- 94°C/1'x40 <b>59°C/1'x40</b> 72°C/1'x40- 72°C/5'

**Table 2.** PrP polymorphism in scrapie affected indigenous goats (*Capra prisca*) [single letter symbolises heterozygous alteration, while two letters (e.g. M/M) symbolises homozygous alteration].

Allele	Codon										five-repeat in 54-102	Allele frequency (%)
	17	22	28	30	127	142	143	154	196	236		
1	M	G	P	P	G	I	H	R	T	I	+	45.45
2	–	–	–	–	–	–	–	L	–	–	+	6.06
3	L/R	–	S/L	–	–	–	–	–	P	P	+	3.03
4	L	–	–	–	–	–	–	–	–	–	+	3.03
5	L	–	–	–	–	–	–	–	–	P	N94G, A96G	3.03
6	R	–	–	–	–	–	–	–	–	P	+	3.03
7	L	–	–	–	–	–	–	–	–	S	D90G, G102W	3.03
8	–	C	–	–	–	–	–	–	–	–	+	3.03
9	–	C	–	–	–	–	–	–	–	T	+	3.03
10	–	–	L	–	–	–	–	–	–	P	V56G, N58G, S59G, I100S	3.03
11	–	–	–	T	–	–	–	–	P	–	W60S	3.03
12	–	–	–	T	–	–	–	–	–	S	G102W	3.03
13	–	–	–	–	–	–	–	–	–	–	Q80H	3.03
14	–	–	–	–	–	–	–	–	–	S	+	3.03
15	–	–	–	–	–	–	–	–	P	M/M	+	3.03
16	–	–	–	–	–	–	–	–	–	M	+	3.03
17	–	–	–	–	–	–	–	–	–	–	Q80H, S81G, C83G	3.03
18	–	–	–	–	–	–	–	–	–	–	84–97 deleted	3.03

## THE EFFECT OF DIFFERENT MANAGEMENT SYSTEMS ON THE OCCURRENCE OF DIARRHEA IN CALVES

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### ABSTRACT

This survey was achieved on 2800 Holliston calves in Tabriz area in Iran. The study examined the effect of calf source, serum immunoglobulin levels, body weight, disinfecting of the navel and the injection of vitamin A in the first day of the life on the occurrence of diarrhea and death in two systems of calf management. Occurrence of diarrhea was significantly influenced by calf source ( $P<0.01$ ), serum immunoglobulin level ( $P<0.01$ ), body weight ( $P<0.05$ ), disinfecting of the navel ( $P<0.01$ ) and the injection of vitamin A in the first day of the life ( $P<0.05$ ) under both management systems. Deaths were significantly influenced by serum immunoglobulin levels and body weight under both systems. Calf body weight among calves which had diarrhea was lower. The occurrence of diarrhea and death was lower among calves group fed acidified milk replacer than calves fed normal milk replacer under similar circumstances.

**Keywords:** management, diarrhea, calf

### INTRODUCTION

Diarrhea remains an important cause of illness and death of young calves. The economic effects of calf scours can be profound. Some cattle herds annually experience death rates of 5 to 10 percent or greater, sometimes with up to 100 percent of calves being ill (1,10). Economic costs of the disease include loss of performance, mortality, and the expense of medication and labor to treat sick calves. In addition, herd owners and their employees often become disheartened after investing long hours to treat scouring calves during an already exhausting calving season(6).

The diarrhea and other clinical signs seen with the disease are caused by the interaction of any of several possible infectious causes and predisposing factors such as lack of colostrum, failure to absorb colostral antibody, poor nutrition and environmental affects.

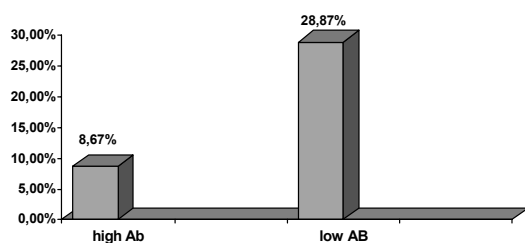
Management systems can have a profound effect on the health of cattle (2, 10, and 11). Our objective was to study the effect of different management systems on the occurrence of diarrhea in calves in Tabriz area in Iran. An effective contact is an exposure to pathogens of a dose-load or duration sufficient to cause disease. Effective contacts can be prevented by physical separating animals, reducing the level of exposure (e.g. through the use of sanitation or dilution over space), or minimizing contact time. These actions have been successfully applied in calf hutch systems to control neonatal diseases in dairy calves (9).

## MATERIALS AND METHODS

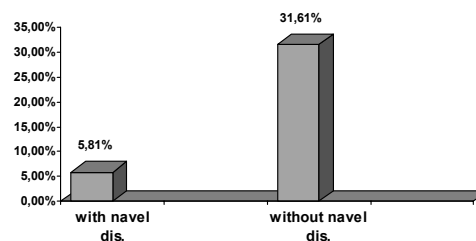
Ten dairy herds in Tabriz area in Iran were monitored for 2 years to determine the effects of environment and management on mortality in preweaned calves. In this time 2800 Holliston calves were studied. 750 calves were with diarrhea. Environmental factors were evaluated by veterinarians during monthly visits to the herds. Management procedures were measured through the use of a questionnaire administered near the end of the project. Mortality in preweaned calves was calculated for each herd by using data from project records on calf mortality and animal inventory, which were collected monthly by veterinarians. Relationships between the management/environment variables and calf mortality were examined by use of analysis of covariance. Herd size, days on a nipple feeder, navel disinfection, type of housing, and whether each calf observed with diarrhea was treated with antibiotics were the variables that had an impact on herd mortality. These variables explained approximately 39% of the variation in mortality among herds.

## RESULT

In this survey 2800 calves in ten dairy herds were studied that 750 calves (37.5%) were with diarrhea. The diarrhea was higher in the calves that had high levels of immunoglobulin in serum than calves with low level it (8.67% and 28.83%, respectively). Total calves divided in 3 groups on the bases of the body weight (20–30 kg, 30–40 kg and 40–50 kg). In these groups the incidence of diarrhea was 21.74%, 9.94% and 5.82% respectively). The calves that were brought from mothers with best body condition had low diarrhea. Disinfection of the navel cause decreasing of the incidence of diarrhea. In the calves that the navel was disinfected early after the birth had low diarrhea from these were not disinfected (5.89% and 31.61% respectively). In the calves that had been used vitamin A (injection) in the firth day, incidence of diarrhea was 14.43% and in these that had not been used it was 23.07%. In the calves that had been used acidified milk replacer the incidence of diarrhea was lower than calves with normal milk replacer (15.21% and 22.29% respectively).

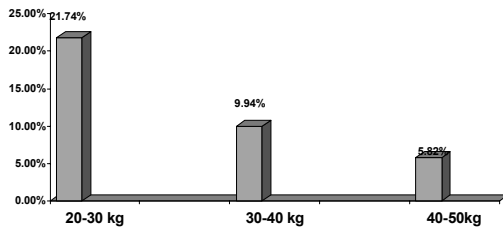


**Graf 1.** The present of the incidence of diarrhea in calves with high and low levels of immunoglobulin in the serum

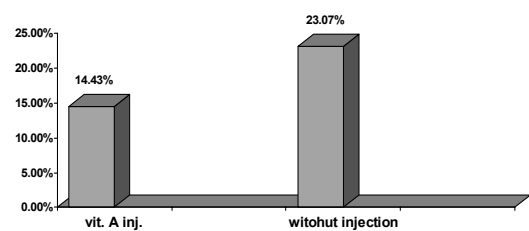


**Graf 2.** The present of the incidence of diarrhea in calves with disinfection of the navel and without it

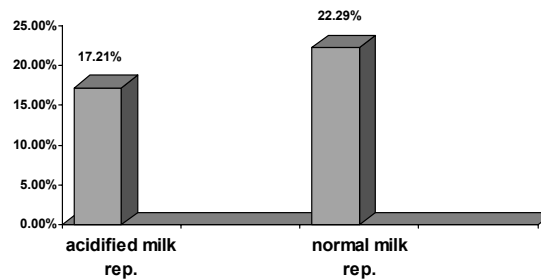




**Graf 3.** The present of the incidence of diarrhea in calves on the bases of the body weight in the birth time



**Graf 4.** The present of the incidence of diarrhea in calves with injection of vitamin A and without it in the early life



**Graf 5.** The present of the incidence of diarrhea in calves on the bases of the use of acidified milk replacer and normal milk replacer

## DISCUSSION

Neonatal calf scours is a multifactorial disease. Agent, host, and environmental factors play important roles in the occurrence of disease and knowledge of these factors become the basis for management intervention to control the disease. Numerous infectious agents have been recovered from scouring calves (5,8). Common agents of neonatal calf scours include bacteria such as *Escherichia coli* and *Salmonella*, viruses such as rotavirus and coronavirus, and protozoa such as cryptosporidia (6,11). Bovine rotavirus, bovine coronavirus and cryptosporidia are ubiquitous to most cattle populations and can be recovered from calves in herds not experiencing calf diarrhea. Further, multiple agents can be recovered from herds experiencing outbreaks of calf diarrhea suggesting that even during outbreaks more than one agent may be involved. Finally, it is important to recognize that the cow herd serves as the reservoir of pathogens from one year to the next (10).

Occurrence of diarrhea was significantly influenced by calf source ( $P < 0.01$ ), serum immunoglobulin level ( $P < 0.01$ ), body weight ( $P < 0.05$ ), disinfecting of the navel ( $P < 0.01$ ) and the injection of vitamin A in the first day of the life ( $P < 0.05$ ) under both management systems. Deaths were significantly influenced by serum immunoglobulin levels and body weight under both systems. Calf body weight among calves which had diarrhea was lower. The occurrence of diarrhea and death was lower among calves group fed acidified milk replacer than calves fed normal milk replacer under similar circumstances.

Because the physiology of the bovine placenta prevents transfer of maternal serum immunoglobulins to the calf before it is born, the neonatal calf is entirely dependent on colostrum immunoglobulins for protection from disease. Calves acquire passive immunity against the common agents of calf scours after absorbing antibodies from colostrum or colostrum supplements. The quality and quantity of colostrum ingested largely influences the level of passive protection. The presence of the antibodies in colostrum directed against specific agents requires prior exposure of the dam to the agent(2,4,and 7).

In heavily used calving grounds, the navel of newborn calves should be treated with iodine. Disinfection is very important in controlling the accumulation and spread of disease-causing microorganisms. Review procedure and have a fluid therapy program prepared for scouring calves, as dehydration and secondary disease problems are the big calf killers(6).

Many calves will also benefit from a vitamin A injection. Vitamin A deficiency disassociated with scours. The calf should be given 500,000I.U vitamin A early in life(10).

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## HEALTH OF GOATS AND MILK QUALITY

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### SUMMARY

It is established that nematodes invasion of digestive tracts affects quality and quantity the animal products. The subjects of this research are quality parameters of digestive tract. Eksperiments were conducted on the farm of goats "Līcīši" in Jelgava region.

Nematodes were detected by MacMaster method.

Goats were divided in to two groups: animals with and without nematode invasion.

Milk samples were tested for ure, cholesterol, amino acids level and fat content on regular basis.

Milk was sampled for several times. Urea, cholesterol and amino acids were estimated, and their relationships were compared in the infected and noninfected goats. It was stated that the invasion of nematodes in milking goats influence both blood haematological and biochemical parameters in many aspects, as well as resulted in the changes of some milk quality indices.

**Keywords:** goats, enteroparasitocenosis, haematological, biochemistry, milk quality

### INTRODUCTION

Obtaining of the qualitative production of the animal kingdom depends on many factors. One of the most significant factors is the correct feeding of goats, keeping and prevention of various diseases. As it is known, goats often suffer from the digestive organs' strongylate invasion. There are many investigations on the digestive organs' parasites bad influence upon the quantity of the milk production to goats but there are little data in literature about the goats' milk quality. It is known that at big digestive parasite invasions, the blood haematological and biochemical indicators change first to animals as well as the mucuous membrane of abomasum to ruminants has been damaged seriously. In cases of parasitosis the eosinophile leucocytes and lymphocytes in blood react first ( Scott et. al., 1998; Balic, 2000; Hertzbergs 2000; Jasmera, 2007). In case of the intensive parasite invasion of digestive organs the changes in the composition of blood protein are being observed as well (Smith, Sherman, 1994; Balic, 1999).

The curative qualities of goat milk are generally known. Goat milk is especially advisable to little children who suffer from allergia. It is an irreplaceable product for people who cannot use cow milk as food (Sprūzs, 1996; restani et. al., 1999; Madsen et al., 2003; McCullough, 2003).

What concerns to separate milk biochemical indicators, then there should be marked the urea amount in milk. Milk urea amount gives possibility to evaluate and control the level of protein feeding to animals. High urea content in milk indicates to the intensified nitrogen educing with urine, the residue of protein or the disparate sugar content in forage as well as it gives evidence of the high destroyable protein content in the rumen. But low urea content in milk gives evidence about an insufficient amount of protein in the forage (Osītis, 2005). It is known that the urea content in milk changes during 24 hours, it is connected with the number of feeding times and the

quality of the forage. The urea content in milk reflects its urea content in blood what the animals have had during the last 12 hours (Madsen et. al., 2003; Ositis 2005). If the protein content in milk is more than 3% but at the same time the amount of urea in milk is lower than 12 mg/100ml, then the conclusion can be made that there is low destroyable ingested protein content in the rumen, there are little carbohydrates in the forage ration and the residue of energy has been observed (Ositis, 2005). We have not succeeded to find an investigation if or how the digestive organs' strongylates of goats influence the milk quality obtained from them.

### MATERIALS AND METHODS

Investigations have been carried out in autumn on one of the biggest goat farms of Latvia – p/f “Līcīši”. During the investigations the goats had been kept in the goat-shed and fed by hay, carrots and oats. Firstly all the milk goats of the farm had been taken the coprological samples and examined according to MacMaster's method (Hoste, 2001). For the further investigations there had been selected 20 goats which had been ascertained the strongylate invasion of digestive organs – 453 eggs per 1 g of faeces as well as 20 goats without the strongylates of digestive organs.

It should be marked that the strongylates of breathing organs had not been ascertained to groups of goats. In the investigations only clinically healthy animals had been used.

Haematological and biochemical samples of blood had been taken from the both groups of goats for several times as well as the samples of milk. Haematocrite (PCV), the number of erythrocytes, the amount of haemoglobin, the number of leucocytes and the leucocytary formula had been determined in the blood haematological samples. Glycose, urea, creatinine, total bilirubin, cholesterol, crude protein, albumin, albumin – globulin ratio, ASAT ferments and the alkaline phosphatase had been determined in blood biochemically. In the samples of goat milk there had been determined the fat content, the amount of protein, the amount of urea, as well as 17 amino acids. The statistical processing of the examination results of the blood haematological, biochemical and milk samples was carried out by the help of Mc Excel programme. The average arithmetical value of each blood biochemical indicator as well as the standard deviation had been estimated. In order to compare and evaluate the changes of blood indicators between the investigation groups of goats, for comparing the two sample sets' dispersion of F-tests and for comparing the average of the two sample sets of T- tests with equal and different dispersions (Arhipova et al., 1998)

### RESULTS

The results showed that the invasion of the digestive organs of goats with strongylates essentially influences the blood morphological and biochemical indicators. (Table 1).

**Table 1.** The change of blood morphological and biochemical parameters of goats with and without invasion

Parameters	Goats with invasion 453 eggs per 1 g faeces	Goats without invasion	Value – p
Eosinophils, %	10.67 ± 3.98	2.67 ± 0.82	p < 0.01
Band. neutrophils, %	4.08 ± 1.62	2.00 ± 1.26	p < 0.01
Albumin, g/l	39.83 ± 4.43	35.67 ± 3.61	p < 0.01
Albumin/globulin coefficient, g/l	1.19 ± 0.24	0.93 ± 0.15	p < 0.01

From the investigated blood haematological indicators in the leucocytary formula the amount of eosinophile leucocytes changed statistically credibly – their number increased from 2.67% to 10.67 as well as the amount of band neutrophil leucocytes – from 2.00% to 4.08%. As it is known, the amount of eosinophile leucocytes has always been increased in cases when there is parasitary invasion in the body of animals (Smith, Sherman, 1994; Balic, 2000). What concerns to band neutrophil leucocytes then they from the moment of birth till the age of six weeks can constitute approximately 2.5% from all neutrophil leucocytes to goats but the band neutrophil leucocytes in the leucocytary formula can even not to be to grown up animals (Jasmer et. al., 2007). Obviously, the strongylate invasion of the digestive organs of goats causes certain intensified developing process of neutrophil leucocytes.

The amount of albumin – from 35.67 to 39.83 g/l had increased statistically credibly (p<0.01) from the investigated biochemical indicators in the blood, albumin globulin ration changed as well from 0.93 to 1.19. It is known that albumin is the main component of the blood serum protein what forms from amino acids in the livers and helps to keep unchangeable osmotic pressure in the body (Lazzarro, 2005). It has been proved that in cases of hard strongylate invasions of digestive organs, the protein level in the blood serum falls (Smith and Sherman, 1994; Balic, 1999). In our case the strongylate invasion of digestive organs to goats was quiet insignificant. Obviously, therefore the body with such a level of invasion at the beginning has quiet successfully coped with the level of albumin in blood is insignificantly but statistically credibly increased. Besides, it has been shown, that if there is sufficiently provided protein in the forage having been fed to animals, then the losses of albumin in blood may also not occur (Smith, Sherman, 1994; Simpson, 2000).

Analysing milk samples from goats which had been invaded by the strongylates of digestive organs and from animals without such invasion we ascertained that the fat % (p>0.05) to the invaded animals had increased a little (Table 2).

**Table 2.** The change of milk parameters of goats with invasion and without invasion

Parameters	Goats with invasion 453 eggs per 1 g faeces	Goats without invasion	Value – p
Fat amount, %	5.5 ± 1.5	4.5 ± 0.9	
Protein amount, %	4.2 ± 0.8	3.3 ± 0.4	p < 0.01
Urea amount, mmol/l	7.1 ± 1.8	4.7 ± 1.6	p < 0.01

What concerns to the protein content in milk, then it statistically credibly (p<0.01) increased – from 3.3% to 4.2%. Just so the amount of urea in milk (p<0.01) being got from the invaded animals statistically credibly increased– (from 4.7± 1.6 to 7.1±1.8).

Analysing the indicators of protein and urea in goats' milk, it can be accepted that there has been an insufficient amount of the micro-organism destroyed protein in the rumen (Table 3). The work in this direction is going on.

**Table 3.** The change of milk protein of goats with invasion and without invasion

Parameters	With invasion 453 eggs per 1 g faeces	Without invasion	Value - p
Lysine	2.47 ± 0.55	2.07 ± 0.29	p < 0.01
Histidine	0.83 ± 0.19	0.71 ± 0.24	p < 0.05
Arginine	0.99 ± 0.22	0.83 ± 0.18	p < 0.05
Apargine acid	1.54 ± 0.34	1.34 ± 0.34	p < 0.05
Treonine	1.48 ± 0.33	1.23 ± 0.28	p < 0.05
Serine	1.25 ± 0.41	1.16 ± 0.26	p > 0.05
Glutamic acid	7.27 ± 1.67	5.78 ± 0.36	p < 0.01
Proline	1.29 ± 0.29	1.14 ± 0.30	p > 0.05
Glycine	0.58 ± 0.13	0.48 ± 0.14	p < 0.05
Alanine	0.84 ± 0.18	0.70 ± 0.20	p < 0.05
Scystine	0.52 ± 0.13	0.43 ± 0.08	p < 0.01
Valine	1.41 ± 0.32	1.22 ± 0.31	p < 0.05
Methionine	0.98 ± 0.29	0.87 ± 0.29	p > 0.05
Ioleicine	1.68 ± 0.81	1.33 ± 0.31	p > 0.05
Leucine	2.06 ± 0.44	1.80 ± 0.38	p < 0.05
Tyrosine	1.10 ± 0.24	0.93 ± 0.22	p < 0.05
Fenilalanine	1.91 ± 0.42	1.69 ± 0.29	p < 0.05

## CONCLUSIONS

1. The strongylate invasion of digestive organs essentially (p<0.01) influences the blood morphological and biochemical indicators of goats. Eosinophile leucocytes increased from 2.67% to uninvadated animals to 10.67% to the invadated ones but the band neutrophil leucocytes correspondingly from 2.00% to 4.08%. Albumins in the blood serum increased from 35.67 g/l to 39.83 g/l but the ratio of albumin globulin – from 0.93 to 1.19.
2. Protein in the goats' milk to the uninvadated animals increased from 3.3% to 4.2% to the invadated animals but the amount of urea in milk increased correspondingly from 4.7 mmol/l to 7.1 mmol/l.
3. For goats with a low stage of invasion at the beginning there is a tendency for increasing of the amount of all amino acids determined in the investigation.

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## RESEARCH OF PHYSICAL AND CHEMICAL ENVIRONMENTAL FACTORS AND THEIR INFLUENCE UPON COWS KEPT AT COLD COW-HOUSES

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**Keywords:** Cow comfort, microclimate, welfare, productivity

### INTRODUCTION

It is known that just from animals which are kept in healthy and good conditions it is possible to get qualitative and high-quality production. Lately very important is welfare of dairy cows regarding the export of production joining the European Union. The welfare is connected both with the cow keeping, feeding, care and milking and provision of microclimate in cattle-shed. The cows shall be fed with an appropriate daily portion regarding their physiological condition, shall have access to appropriate clean, drinking water regarding to sanitary rules. The care of the cows shall not raise too high stress, make pain and traumatism. The indicators of microclimate in cattle-shed (air humidity, temperature, gas concentration, ventilation frequency, amount of dust, light etc.) shall promote physiological process of cows (2, 4, 6). There are used different solutions in cow keeping. If 20–30 years ago it was dominant to leash the cow in European countries, then lately more popular is to not to leash the cows in boxes, kombi-boxes, on sloping floor or deep litter. It is possible to keep the cows in so-called cold cattle-sheds, whose construction costs are less than 20–50% (3).

It is necessary for animals to have a lot of fresh and dry air. That is the reason why the premises shall be with the appropriate capacity and good ventilation. The cows are not disturbed because of low weather temperature (around 0°C) or fresh air flow, but humid air and draught is dangerous. It is important to assess the quality of air in appropriate place, where the cows are sleeping or standing. The initiators of illness are bred in warm and humid premises; meanwhile the cows are under the stress (1, 2, 4, and 5). Due to unfavourable changes of microclimate, the productivity of animals can be decreased by 20 = 30% as well as animal protection against diseases can be decreased. (2). It is observed that the cows which are constantly in places with 30 mg/m<sup>3</sup> or higher ammonia concentration in the air of cattle-shed can have even anaemia, decreased reserve of blood alkali and bad usage of nutrients. As a result milk yield is dramatically decreased.

The cattle-shed No 1 of dairy cows shall be planned for 106 cows in unleashed keeping 2.20 m x 1.20 m in boxes with feeding rack, parturition zone and place where to keep calves of different ages. There is milking parlour with fir-tree shaped milking hardware, milk cooling device as well as the staff room situated in the middle of long wall in the cattle-shed. Reinforced concrete construction columns of the moving parts in cattle-shed and folding metal ceiling framework. External walls are made of aerated concrete panels, but the terminal wall of the building are made of ceramsite blocks. The stone-wool shall be used in order to insolate the ceiling. The floor within the zone of feeding and droppings aisle made of concrete, but box sleeping-places as litter is used coarse sand, which is added of necessity around twice a week.



There shall be built a special herringbone type milking parlour for 2 x 5 cows for milking. There the cows are situated next to each other obliquely against the trench. For milking places on the one side of trench there are common entrances and exit gates, therefore the cows in milking places are let in and out by groups.

Drinking bunks (one per 15 cows) shall be situated at the end of box rows in order to water cows.

Dung shall be brought out with tractor machinery once a day. Draining canals and slurry well shall be installed for collecting slurry. Ventilation system of tubes with pipes of exhaust fumes shall be functioning within the farm. However, fresh outdoor air shall enter through windows and specially installed opening in the cornice part. The artificial light luminescent electric bulbs – 2 x 36 W situated in 3 groups; 11 bulbs in each. During the night – pilot light (blue light 40 W bulbs).

Stall No 2 of milking cows with the way of tied keeping is constructed in 1975 as a complex of milking cows with finished production cycle for 400 cows. There are 200 milking cows situated in isolated block in stall. The construction of cattle-shed shall be columns of reinforced concrete with folding ceiling framework of reinforced concrete metal. External walls shall be built of bricks in which there are designed main doors. There is automatic water bunk in the stall – one bunk where to drink per two cows. Each cow row has its own vacuum-tube and milk tube. Withers bar for cow fixation shall be in height of 1.05 m from the ground. Corrugated ceramic tiles shall cover the floor in stall. Saw dust and straw are used as litter. Tractor and food-distributor are used for mechanization of labour.

16 airshafts shall be installed for output of cattle-shed air. However, fresh air shall enter through windows and specially installed opening in the cornice part. Condensate on windows and metal constructions shows that air exchange (ventilation) is insufficient. Artificial light is installed in three rows and 10 incandescent bulbs in each of row, but incandescent bulbs often blow out and are not replaced in time, therefore does not assure artificial light 4.5–5 W by m<sup>2</sup> regarding the appropriate standards.

Dung removal and stall cleaning shall be made twice a day with rake-conveyer attached to articulated tractor; afterwards it shall be transported to midden. Slurry shall be drained to slurry storage, where it is pumped out of necessity.

The mode of animal feeding, amount of daily food and composition in both of farms are similar. Increasing relative air humidity over 80%, daily milk yield of highly productive cows on every of 5% humidity increase, decreases by 1.2–1.4 kg (4). The major works in maintaining microclimate are bringing out the dung, stall strewing, ventilation of premises, cleaning and preventive disinfection.

In order to maintain optimal indicators of microclimate and to make appropriate milk yield, the air quality of cattle-shed shall be regularly assessed and air composition shall be tested.

The objective of the research is to identify some indicators of microclimate regarding milk yield in cow stalls with different ways of keeping – leashed or unleashed.

## CONTENT AND METHODOLOGY

The research in both farms is made between 1<sup>st</sup> of November 2003 – 1<sup>st</sup> of May 2004. Cows were fed and milked similarly in both farms twice a day at 5.00 AM and 15.30 PM. Agricultural consultations and software “LEDA” from education centre were used in order to make daily food dose. The indicators of microclimate (air temperature of premises, air motion speed, air humidity and gas structure) were defined during the research in farms No 1 and No 2 36 times in each one

taking into consideration existing methodology. However, the equipment was used from the laboratory of Food and Environment Hygiene Institute. Milk yield was counted every day. Statistical functions, data analysis tools, correlation and regression functions of MS Excel were used for the analysis of gained results.

## RESULTS AND DISCUSSION

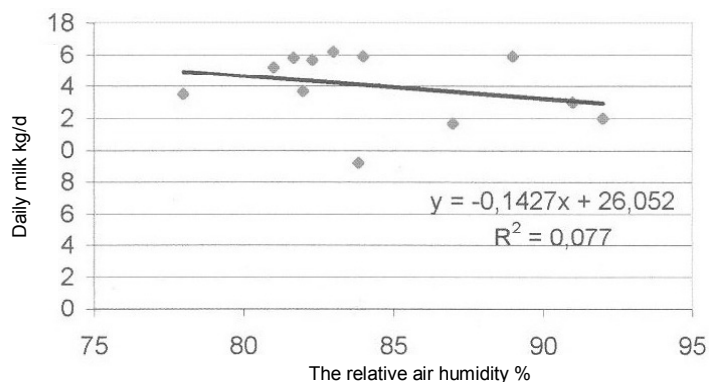
Results of the research verified that daily milk yield are different in accordance with similar feeding conditions and food doses where there are different ways of keeping cows (leashed and unleashed). Daily milk yield keeping cows unleashed was 14.0–17.3 litres in the farm No 1. However, in the farm No 2 where the cows were kept leashed daily milk yield changed to 9.2–14.9 litres. It is known that the milk yield is affected also such factors as breed, lactation, parturition etc., therefore the connection between the milk yield and the indicators of microclimate was determined together in both cattle-sheds.

The average indicators of microclimate of cow premises in comparison with the norm are seen in the Table 1.

**Table 1.** Indices of microclimate in the cow sheds of Farm No1 and Farm No 2 in winter 2003/2004

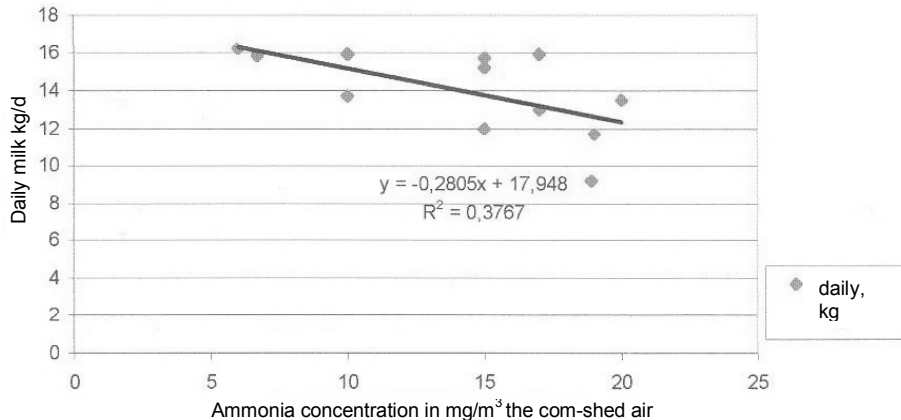
Indicators	Farm Nr 1	Farm Nr 2	Norm
Relative air humidity %	74–88	73–94	75–85
Conc. of ammonia mg/m <sup>3</sup>	4–17	10 – 25	<20
Air motion m/s	0,2–0,35	0,02–0,25	0,3–0,4
Air temperature, °C	1,0–11,5	6,0–19,5	8–12

Evidently the relative air humidity in the farm No 1 ranged between 74–88% which complies with the advised standards overall. The relative air humidity in the farm No 2 increased over the standards by 94% in December, January and February that could be connected to insufficient air motion speed due to the deficient ventilation. Relative fluctuations of humidity in the air of cattle-shed did not affect a lot daily milk yield in research (Figure 1).



**Figure 1.** The relative air humidity % in the cowshed air on the daily milk yield in farm 1 and farm 2

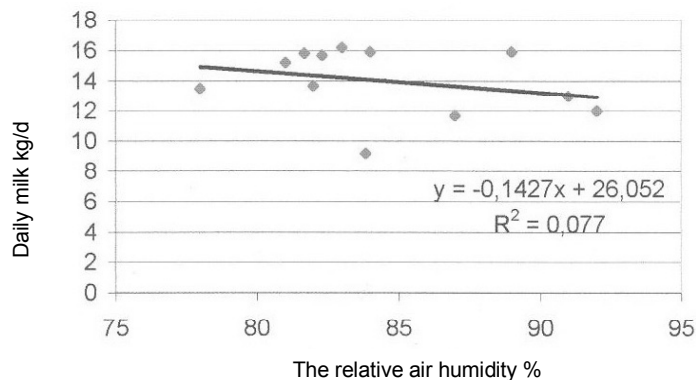
Ammonia concentration in the cow farm No 1 was between 4 and 17 mg/m<sup>3</sup>, i.e. according to the norm (< 20). Also in the farm No 2 in the majority of research it was within the norms with fluctuations from 10–25 mg/m<sup>3</sup>. The highest ammonia concentration in the farm 20–25 mg/m<sup>3</sup> was registered in April that is above the norm. As the ammonia concentration in the air of cattle-shed was increased, decrease of milk yield has been registered (Figure 2).



**Figure 2.** The ammonia concentration mg/m<sup>3</sup> the cowshed air on the daily milk yield in farm 1 and farm 2

Air motion speed in the farm No 1 was corresponding to the norm (0.3...0.4m/sec) and ranged from 0.2–0.35 m/sec.

However, in the farm No 2 it was considerably insufficient especially in January and February (0.02ficient. It has been noticed that on the moment when there is low air motion speed daily milk yield of cow is a little bit lower (Figure 3).



**Figure 3.** The relative air humidity % in the cow shed air on the daily milk yield in farm 1 and farm 2

Temperature of the cattle-shed in the stall No 1 in several measurements in December and especially in February was above the advised norm (8–12°C) and ranged from 1.0–11.5°C, but in the stall No 2 from 6.0–19.5°C, which exceeded the optimal at the end of March and April. These fluctuations match to the fluctuations of the outdoor air temperature. Taking into consideration observations the cattle-shed indicators of the air temperature did not affect milk yield a lot. The presence of hydrogen sulphide in both places was not found.

## CONCLUSIONS

1. The Indicators of microclimate (relative air humidity, ammonia concentration, air motion speed, cattle-shed air temperature) in the farm No 1 with unleashed cows was corresponded more to the norms in force as in the farm No 2 where the cows were leashed.
2. Daily cow milk yield in the farm No 1 was higher than in the farm No 2. There has been observed a link between the indicators of cattle-shed microclimate – ammonia concentration, air motion speed and daily cow milk yield. Increased ammonia concentration and decreased air motion speed demonstrate negative influence to the cow milk yield.
3. Correlation between relative air humidity and yield in cattle-shed as well as air temperature and yield was not found in research.

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## EPIDEMIOLOGY AND ECONOMICS OF BRUCELLOSIS IN ANIMALS AND ITS ZONOTIC SIGNIFICANCE

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### ABSTRACT

Brucellosis is endemic in animals in India caused by mainly *Brucella abortus* and *Brucella melitensis* and is readily transmissible to man as an occupational hazard. Active surveillance programme since last 20 years revealed the 9.3% positive reactors of 13396 cattle tested in organized and unorganized farms through out the country. Besides, *Brucella abortus* biotype- I has been isolated in 11% of 371 aborted bovine fetuses. In order to assess the transmission of Brucellosis from infected cattle to man, at total 119 sera of in contact persons working in cattle farms were tested and of these 34.4% was found positive for Brucellosis. A total of 7880 breedable cows and bulls were tested for the presence of *Brucella* antibodies and 980 (12.4%) bulls were found positive for Brucellosis. In order to control the Brucellosis in organized farms, test and segregation policy is being adopted and seropositive animals are kept away from the main farms under ADRIF concept, which is 'Animal Disease Research and Instructional Farm' meant for teaching and research purpose and maintained under strict biosafety conditions. Since adoption of the control programme, incidence of Brucellosis reduced dramatically to merely 1.2%. Based on the Epidemiological data of active surveillance programme, it is estimated that due to brucellosis, there is a loss of US\$ 58.8 million per year in India.

### INTRODUCTION

Brucellosis is one of the world's major zoonoses, which is endemic in India caused mainly by *Brucella abortus* and *B. melitensis* and is readily transmissible to man as an occupational hazard. The disease in cattle seems to be associated primarily with intensive farming practices in large organized animal farms. (Smits and Kadri 2005). Brucellosis is widely prevalent in India among the bovine population both in farms and in the villages. It causes heavy economic loss to the animal industry through abortion, delayed conception, temporary or permanent infertility in the affected animals. Based on the epidemiological data of active surveillance programme, it is estimated that due to Brucellosis, there is a loss of US\$58.8 million per year in India. The present work is epidemiological investigations including isolation of *Brucella spp.* testing of serum samples and adoption of control measures.

### MATERIALS AND METHODS

Samples were collected from 371 aborted cows, included parts of placenta, vaginal swabs, and samples from aborted fetuses (abomasal contents, heart blood, and peritoneal cavity fluid) as well as paired serum samples from affected animals. Isolation was attempted by following the method

of Alton et al. (1975). In present investigation, a total of 13396 cattle serum were collected from both organized and unorganized farm through out India. Serum samples were collected from a total of 7880 breedable cows from organized herds in the country having mainly crossbred population. Serum samples were also collected from 119 cattle farm workers including veterinarians, paravets, attendants etc.

Serum samples were tested by Rose Bengal Plate Test (RBPT; Alton et al., 1975), standard tube agglutination test (STAT; Alton et al., 1975) and enzyme linked immunosorbant assay (ELISA; Nielsen and Wright, 1984) employing standard procedures. The standard methods for epidemiological analysis were followed as per Thrusfield (1998).

## RESULTS & DISCUSSION

Brucellosis is widely prevalent through out India among the bovine population both in farm and in the village animals (Polding, 1943, 1947; Vishwanathan, 1944; Mathur, 1963, 1964). Public health significance of Brucellosis is well known (Koshi and Myers, 1969). The disease has an added importance in countries like India where the conditions are conducive to wide spread human infection on account of unhygienic conditions and poverty. Present study revealed that out of 119 sera of cattle farm personnel 34.4% had the *Brucella* antibodies, which was due to the fact that they were constantly exposed to infection while milking, attending to cows during parturition and other complications. In the present study *B. abortus* biotype I was isolated from 41 (11%) clinical samples out of 371-aborted fetus. *B. abortus* biotype I is common in organized farm, while *B. abortus* biotype III is common in the villages (Yadav, 1988).

Bulls can act as a source of Brucellosis because they excrete the organism through their semen. In the present study a total of 12.4% bulls were found seropositive to the disease. The culling and segregation of positive bulls is necessary to control Brucellosis.

In India, effective control of brucellosis is a national problem. A major obstacle in the control of this disease has been the disposal of the positive animals. In brucellosis free countries, test and slaughter of positive animals is proved effective. However, in India the existing socioeconomic conditions do not advocate this policy. The alternative method of "test and segregation" is perhaps the only method, which is practical and feasible in our country.

Control programme comprised periodical testing of all animals in each farm and removal of the positive reactors immediately till no reactor animals are found. Disease free herds were treated as "closed herds". The seropositive cattle are kept in a separate farm called ADRIF (Animal Disease Research and Instructional Farm), which is situated away from the main farm. This resulted in a reduction of incidence to merely 1.2%. ADRIF also served the purpose of teaching and research under strict biosafety condition (Ann, 2006). Similarly by adopting this method it was possible to reduce the overall incidence of brucellosis in UttarPradesh from 5.6 to 1.42% in 8 years (Pathak, 1966). Likewise in Haryana, as a result of half yearly testing and segregation of reactors on the same farm, the percentage of the positive reactors had been brought down from 25 to nil in 5 years (Sharma, 1965). To get the disease under control, it is recommended that even in the closed herds animals must be tested at regular intervals to detect 'sub-clinical carriers' and remove wherever necessary. Other important steps are provision of housing for animals, arrangement for segregation of aborted and infected animals, proper disposal of aborted materials and disinfections of contaminated premises besides calf hood vaccination.

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## THE PREVALENCE OF CLAW DISORDERS AND THEIR EFFECT ON MILK YIELD IN HUNGARIAN DAIRY HERDS

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In the present study we have collected data on the prevalence of claw disorders in Hungarian dairy farms and studied the connection between severity and milk production.

Data collection was carried out on randomly chosen dairy farms at the time of the regular claw trimming from the spring of 2005 to November, 2005. 37–120 cows from different production groups per farm were studied, giving a total of 540 animals.

During claw trimming we have collected data on the prevalence of digital dermatitis (DD), interdigital dermatitis (ID), laminitis, sole ulcer, heel-horn erosion (HHE) and interdigital hyperplasia (IH). In case of DD we have recorded the localization and extension of the lesion. Data on the calving date, locomotion score, milk yield, milk fat, milk protein content and SCC of the studied animals were also collected.

A total of 1581 claws were diagnosed with disorder. DD was found most prevalent (making up 45,9% of all disorders, affecting 33,5% of all claws, Figure 1). It could also be concluded that hind claws are more likely to be affected by different disorders (except for laminitis and heel-horn erosion). Dermatitis digitalis was present on 5 predilection areas, out of which the plantar area was affected in most of the cases (81% of all DD cases, Figure 2).

Merely 13 cows out of all studied animals were given a locomotion score 1, meaning that only 2,4% of all animals were not lame. It was observed that lower locomotion scores correlated with lower numbers of disorder/claw and smaller DD lesions (Figure 3 and 4). It was also found that cows suffering from DD or laminitis are more likely to present other disorders at the same time and be given a higher locomotion score (Table 1 and 2). We have found no significant difference between the milk yield and milk composition of lame and healthy animals.

Results of the present study point out that claw disorders are highly prevalent in Hungarian dairy farms thus prevention of such diseases is greatly important. Figure 2. The prevalence of different DD types



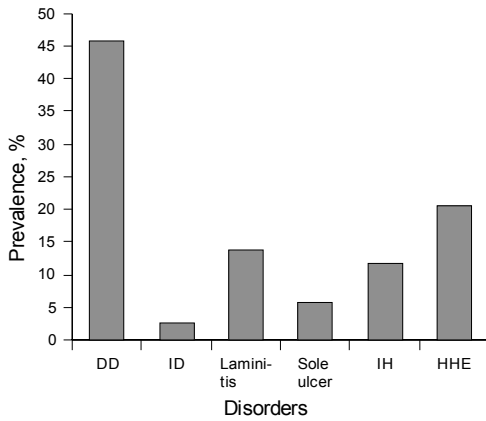


Figure 1. The prevalence of claw disorders

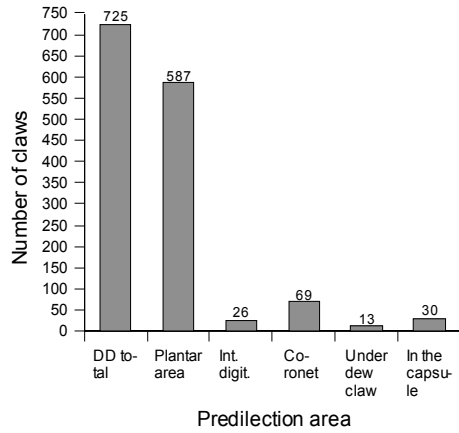


Figure 2. The prevalence of different DD types

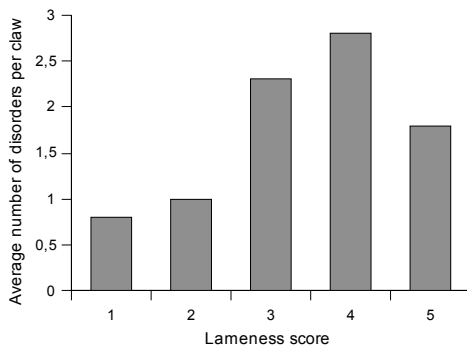


Figure 3. Average number of disorders per claw

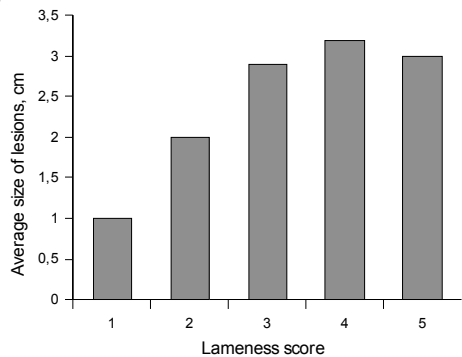


Figure 4. Average size of DD lesions

Table 1. Correlation between DD and certain indices of claw disorders

	<i>DD</i>	
	no	yes
Prevalence of laminitis, %	5,3	12,4
Prevalence of sole ulcer, %	2,6	5,5
Average number of disorder per claw	0,9	2,4
Lameness score	2,3	2,9

Table 2. Correlation between laminitis and certain indices of claw disorders

	<i>Laminitis</i>	
	no	yes
Prevalence of DD, %	19,4	41,6
Prevalence of sole ulcer, %	2,8	11,5
Average number of disorder per claw	1,5	3,2
Lameness score	2,7	3,0

## GENETIC PARAMETERS ASSESEMENT IN A ROMANIAN SWINE POPULATION

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### ABSTRACT

The genetic structure of a population is described by the number of the genotype categories and their frequencies. Regarding the productive characters, being involved a large number of genes with an additive effect, the describing of the genetic structure becomes difficult because it is possible to determine each gene category frequency and the genotype presents a very large reaction field that could not allow the sharing of the phenotypes in perfect distinct classes.

That is why for such features, the describing of the genetic structure of a population is made with the aid of the genetic parameters: heritability, repeatability and genotypic correlations.

Due to the fact that the genetic structure of a population modifies from a generation to other one, it is absolutely necessary to asses the genetic parameter of each generation.

Thus, the aim of our study is to asses the genetic parameter of a L.S.-345 Peris swine population, obtained by testing their own performances, being known their importance for the selection process of the reproductive individuals.

**Keywords:** boar, genetic parameters, heritability, biometric, variance, slight of lean

### INTRODUCTION

For animal breeding, the knowledge of the genetic parameters has a major importance, because they are necessary for giving the priority to the genetic breeding or global animal production increasing, choosing the breeding system, establishing the selection objective and setting up the breeding program.

The correlation between different characters shows indications on the selection range of a character and if it is correct or incorrect these two or more characters be in the same time the breeding objective.

The correlation could be:

- phenotypic, established by the values carried out by quantitative measures;
- genotypic, established by additive variance value;
- Environmental, the environmental factors could influence in the same direction or in different ones, two or more characters.

We know that genetic parameters and the economic values represent an important stage of an animal breeding program setting up and the fact that the consumers prefer a high quality meat, so the swine breeding efficiency depends on it, in this study we wish to give our contribution in the breeding work of a swine population.

## MATERIALS AND METHODS

The population of L.S.-345 Peris is a paternal line of swine, created in a Romanian Research Institute and it represents one of the using ways of pigs' genetic diversity along the whole world, having in view to emphasize the heterozis effect and complementarities between breeds.

In the present, it is used like a genitor population as a terminal sire for obtaining the pig hybrid for the Romanian pork market. The biologic material used in the present study was represented by 1620 tested individuals (young sows and boars) on their own performances, owing to 45 boar families.

For establishing the genetic parameters, there was applied a mix biometric model, a sire model:

$$y_{ijk} = S_i + V_j + e_{ijk}$$

Where:

$y_{ijk}$  = observed performance of k descendent, i sex, owed j boar;

$S_i$  = fixed effect of individual sex;

$V_j$  = compulsory effect of boar;

$e_{ijk}$  = each value error.

The studied characters were:

- the final body weight at the end of testing (carried out by their own growing performances);
- the slight of lean, corrected for 100 kg, carried out by ultrasound device Piglog 105;
- daily average gain (during the whole life);
- age at 100 kg.

Because all the characters were measured on each individual, it was used the canonic transformation of the phenotypic values. For obtaining the observational compounds of genotypic and environmental variances and covariance the results were transformed with the aid of the reverse matrix of the canonic transformation.

The matrix is iterative estimated till it is arrived to convergence. After these, the observational compounds are transferred in causal compounds.

$$V_f = S_F^2 = S_T^2 + S_E^2$$

## RESULTS AND DISCUSSIONS

In table 1 there are presented the average performances of the analyzed population.

**Table 1.** Average performances of the analyzed population

No.	Character	U.M.	N	$\bar{X}$	s	c.v.(%)
1	Body weight at the end of testing	Kg	1623	102,2286±0,1862	7,5024	7,2964
2	Slight of lean (corrected)	mm	1623	11,5946±0,0724	2,9204	25,1880
3	Daily average gain	g	1623	0,5651±0,0010	0,0414	7,3300
4	Age at 100 kg	Days	1623	178,7566±0,2649	10,6738	5,9711

**Table 2.** Presents the observational compounds of variances

Characters / pair of characters	$S_F^2$	%	$S_F^2$	%	$S_F^2$	%
Body weight at the end of testing (A)	63,1695	100	60,5252	95,81	2,6443	4,19
Slight of lean (corrected) (B)	8,5239	100	7,8228	91,68	0,7011	8,32
Daily average gain (C)	26,8055	100	25,6269	95,60	1,1786	4,40
Age at 100 kg (D)	115,5039	100	109,9895	95,23	5,5239	4,77

The observational compounds of the analyzed covariance are presented in table 3.

**Table 3.** Observational compounds of covariances

Couple of characters	$cov_F$	$cov_I$	$cov_i$
<i>Body weight at the end of testing X</i>			
Slight of lean (corrected) 100 Kg	1,8278	0,1940	1,6338
Daily average gain	-0,9493	-0,0403	-0,9090
Age at 100 kg	36,1867	1,7417	34,4450
<i>Slight of lean (corrected) 100 Kg X</i>			
Daily average gain	-0,1472	-0,0114	-0,1357
Age at 100 kg	39,0380	1,8549	37,1831
<i>Daily average gain X</i>			
Age at 100 kg	38,9512	1,8614	37,0899

There were also calculated the values of heritability for the analyzed characters:

**Table 4.** Heritability values of the analyzed characters

Couple of characters	$h^2$	$S_{h^2}$
Body weight at the end of testing (A)	63,1695	100
Slight of lean (corrected) (B)	8,5239	100
Daily average gain (C)	26,8055	100
Age at 100 kg (D)	115,5039	100

Following the data in table three there were analyzed the correlation between the pairs of the studied characters and the results are presented in table5.

**Table 5.** Components

Couple of characters	$r_F$	$Sr_F$	$r_G$	$Sr_G$	$r_e$
<i>Body weight at the end of testing X</i>					
Slight of lean (corrected) 100 Kg	0,0788 <sup>NS</sup>	0,02384	0,1425 <sup>*</sup>	0,07111	0,0662 <sup>NS</sup>
Daily average gain	-0,5958 <sup>***</sup>	0,03138	-0,5781 <sup>***</sup>	0,02861	-0,5986 <sup>***</sup>
Age at 100 kg	0,2918 <sup>**</sup>	0,02090	0,3135 <sup>***</sup>	0,04423	0,2886 <sup>**</sup>
<i>Slight of lean (corrected) 100 Kg X</i>					
Daily average gain	-0,6705 <sup>***</sup>	0,03210	-0,6544 <sup>***</sup>	0,02198	-0,6756 <sup>***</sup>
Age at 100 kg	0,6771 <sup>**</sup>	0,01411	0,5974 <sup>***</sup>	0,02662	0,6936 <sup>***</sup>
<i>Daily average gain X</i>					
Age at 100 kg	0,7000 <sup>***</sup>	0,01360	0,7301 <sup>***</sup>	0,02124	0,6953 <sup>***</sup>

NS = no-nsignificant;

\* = significant differences;

\*\* = distinct significant differences;

\*\*\*= very significant differences;

It could notice that, regarding the genetic correlations, they are very significant for all the studied couples, that denoting the existence of some common genetic mechanisms involved in their determinism, with only one exception, the couple: body weight at the end of testing – slight of lean, where the recorded genetic interdependence is just significant from the statistical point of view.

The phenotypic correlation follows the same trend as the genetic ones, their interdependence being very significant, with the same exception as above. Generally, the genotypic correlation coefficients have values between -0.6705 and 0.7000. Only two of the six phenotypic correlations are negative, the others are significant and positive. From all the correlation, the genotypic correlation is the most important. A comparative study emphasizes the fact that they could not be known upon appearances. Generally, the way that animals are exploited influences very much the genetic value of the individuals. So, it could conclude that their simultaneous breeding is possible and we could introduce both characters in the objectives of the selection.

## CONCLUSIONS

1. The determined values for the analyzed population are typical for a paternal swine population.
2. The high variability coefficient of the character slight of lean shows the fact that in this population there is a high variability for this character, so it could apply a severe selection for its improving.
3. In the analyzed population, the values of the heritability coefficients frame the most characters in the category of the easy heritable ones.
4. The estimated heritability values for the body weight at the end of the testing and the age at 100kg, does not recommend the applying of the selection based on their own performances, being necessary other information sources. It could recommend the applying of the combined selection index.

5. The intense negative correlation between the slight of lean and the daily gain show the fact that in the analyzed population the average gain is realized mainly on the base of muscular tissue growth and less on fat depositing, a specific fact for the paternal population.
6. The genetic positive and significant genetic correlation between the slight of lean and the age at 100 kg, represents a benefit fact, because the improving of the speed of growth and the decreasing of the age realized two important goals: the economic efficiency and the main wish of the consumers and the processors to obtain leaner carcasses.

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## BOVINE MASTITIS CAUSED BY COAGULASE-NEGATIVE STAPHYLOCOCCI MAY PERSIST

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### SUMMARY

Persistence of coagulase-negative staphylococci in intramammary infection during lactation was studied. Milk samples from 328 udder quarters of 82 dairy cows were collected every 4 weeks until the end of lactation. CNS isolates were analyzed with API Staph ID 32 test and genotyped using amplified fragment length polymorphism analysis. In total, 63 CNS infections were detected during lactation. Twenty-nine infections persisted and 34 were transient. Most of the persistent infections lasted until the end of lactation. The SCC was clearly elevated during CNS infection. *Staphylococcus chromogenes* and *Staphylococcus simulans* were the CNS species isolated most often.

**Keywords:** mastitis, bovine, persistent, coagulase-negative staphylococci

### INTRODUCTION

Coagulase-negative staphylococci (CNS) in dairy production are often considered pathogens of minor importance, especially in contrast to *Staphylococcus aureus*, streptococci, and coliforms, which may cause severe mastitis. In many countries, CNS-s are the predominant pathogens associated with mastitis (Waage et al., 1999; Macovec and Ruegg, 2003; Nevala et al., 2004) and isolated in the prevalence studies (Wilson et al., 1997; Pitkälä et al., 2004; Tenhagen et al., 2006). For reasons not yet known, CNS infection is especially common in heifers (Honkanen-Buzalski et al., 1994; Aarestrup and Jensen, 1997). CNS mastitis usually remains subclinical or mildly clinical (Taponen et al., 2006), but affects the milk quality by increasing the somatic cell count (SCC) (Djabri, 2002), and may slightly decrease milk production (Timms and Schultz, 1987; Gröhn et al., 2004; De Vliegher et al., 2005). Although CNS mastitis is commonly expected to cure spontaneously, there is some evidence that CNS infection may persist in the udder for long times or even for the entire lactation (Aarestrup and Jensen, 1997; Laevens et al., 1997a; Chaffer et al., 1999; Taponen et al., 2006).

Our aim was to investigate the persistence of CNS in the udder of lactating cows over the entire lactation period using consecutive sampling and pheno- and genotyping of the isolates. The influence of CNS infection on the milk SCC was also examined.

## MATERIALS AND METHODS

The study was conducted in the research dairy herd of the University of Helsinki. A total of 328 udder quarters of 82 dairy cows (30 primiparous, 52 multiparous) were sampled 2 weeks before calving, at calving, and every 4 weeks thereafter until the end of lactation or until the cow left the herd. CNS isolated from the milk samples were analyzed with API Staph ID 32 test and genotyped using amplified fragment length polymorphism (AFLP) analysis. AFLP patterns were used both for similarity analysis between CNS isolates and for species identification. For the latter, AFLP patterns of CNS isolates and 48 staphylococcal type strains were used as operational taxonomic units in numerical analysis. In addition, the somatic cell count (SCC) of the milk samples was measured during lactation. CNS infection was determined as persistent if CNS growth was detected in at least three consecutive or almost consecutive samplings (one bacterially negative sample was accepted between two samples with growth of the same CNS strain), and the isolates from these samplings possessed identical AFLP patterns.

## RESULTS

CNS infection was detected during lactation in 63 (19.2%) of all 328 quarters. Thirty of these 63 quarters were in 22 primiparous cows and 33 in 25 multiparous cows. Thus, the incidence of CNS infection during lactation was 25.0% for quarters in first lactation and 15.9% for quarters in later lactations. All infections were associated with subclinical or mildly clinical mastitis. Before parturition, at parturition, or both in total 57 quarters were infected with CNS (37.5% of first lactation quarters and 5.8% of subsequent lactation quarters). In 28 of these 57 quarters, CNS infection was detected again during the lactation. Thirty-two quarters were infected at the beginning of lactation and 33 quarters for the first time later during lactation. In cows in first lactation 74.2% of quarters and in cows in later lactations 20.0% of quarters were infected at the beginning of lactation.

CNS infection persisted in 29 quarters (in 13.3% of quarters in first lactation and 6.3% of quarters in later lactations) and was transient in 34 quarters (11.7% of quarters in first lactation and 9.6% of quarters in later lactations). Most persistent infections lasted from the detection of the infection to the end of the lactation or culling of the cow. In 14 quarters with transient infection, the causative strain was isolated twice and in 20 quarters only once.

The mean of geometric means of SCC of quarters with persistent CNS infection was during the infection over 600 000 cells/mL, which is clearly higher than the mean of geometric means of SCC of quarters with no bacterial growth throughout the lactation, which was about 60 000 cells/mL.

According to both API Staph ID 32 test and AFLP analysis, the predominant CNS species both in transient and persistent infections was *S. chromogenes*, followed by *S. simulans*. The API test was unable to identify almost one third of the isolates with an acceptable identification result set at 90% of probability. The agreement in species identification of API and AFLP analyses was 70%.



## DISCUSSION

About half of the CNS infections persisted for long periods during the lactation. This result supports earlier evidence, which shows that CNS cause chronic mastitis, and at least some of the CNS infections persist (Aarestrup and Jensen, 1997; Laevens et al., 1997a; Chaffer et al., 1999; Taponen et al., 2006). Most of the quarters with persistent infections remained infected from the detection of infection until the end of lactation. Based on the AFLP patterns, one clone was often isolated from the quarter before and after calving, but about half of the quarters became infected for the first time later during lactation. Multiparous cows were in general infected with CNS in later lactation, whereas primiparous cows usually were infected already in the beginning of lactation. The same has also been shown by Gröhn et al. (2004). In 41% of the transient infections, the same strain was detected twice, the infection thus lasting at least one month. Approximately 50% of the quarters in which a CNS infection was detected before or at calving were cured spontaneously during lactogenesis.

Differences in persistence between CNS species may exist, but in our data with limited number of infected quarters, such a difference between the species was not detected. The same CNS species and isolates with similar AFLP patterns were found in both persistent and transient infections. This suggests that host-microbe interaction plays a key role in the genesis of infection. Heifers and primiparous cows were much more susceptible to CNS mastitis than were multiparous cows. Although this is commonly known, the reason for this phenomenon still remains unknown.

Quality requirements for raw milk are high and the price of bulk tank milk is often connected with the somatic cell count (SCC) of the milk. In Finland, the requirement for best bulk milk price is SCC <250 000 cells/mL and bacterial count <50 000/mL. The dairy producers pay much attention to keeping the SCC low, and any bacteria persisting in the udder and increasing the SCC are in this respect harmful. Compared with infections caused by other common Gram-positive mastitis pathogens, such as *S. aureus* and streptococci, the SCC in quarters infected with CNS is rather low. It is, however, about 10-fold higher than the SCC of healthy quarters, which typically remains under 50 000 cells/mL (Barkema et al., 1999). We found the SCC of a healthy quarter to remain between 10 000 and 40 000 cells/mL in both first and later lactations, whereas in CNS infections the SCC is clearly elevated. The SCC in quarters with persistent CNS infection considerably varied from sampling to sampling, and so the geometric means of quarters infected with CNS. Compared with the study of Djabri et al. (2002), the mean SCC of quarters with CNS infection was here somewhat higher than the average SCC in that meta-analysis. Even a transient CNS infection caused a temporary increase in SCC, which is consistent with the report of Laevens et al. (1997b). CNS infection induces an immunological reaction in the udder and should not be considered merely teat canal colonization or normal situation for the udder.

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**B2 –  
ANIMAL HYGIENE MEASURES  
FOR THE PROTECTION OF ENVIRONMENT  
AND PUBLIC**



## ORAL PRESENTATIONS

### REDUCING THE CONCENTRATION OF AIRBORNE PARTICLES IN HORSE STABLES

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#### SUMMARY

Horses appear to be more sensitive to airborne particles than other species of livestock and high concentration of airborne pollutants in horse stables reportedly interfere with the health and athletic ability of these animals. In order to develop best practice management procedures, the effects of three different bedding material treatments on the resultant air quality were assessed during an experiment and compared to “standard” sawdust bedding (control). The effects of (1) sawdust impregnated with canola oil, (2) straw bedding and (3) the use of “horse-nappies” on the concentration of airborne particles inside four horse stables were studied, using a 4x4 Latin Square experimental design. The results demonstrated a significant reduction in the concentrations of inhalable and respirable airborne particles in the horse boxes treated with oil-impregnated bedding material. This technique would enable horse keepers to improve the environmental quality of horse stables at a relatively low cost.

**Keywords:** horses, air quality, stables, environment, oil impregnation, bedding material

#### INTRODUCTION

Adequate air quality, including low airborne particle concentrations in stables is an important component of good horse husbandry (Blunden *et al.* 1994; Carpenter 1986; Woods *et al.* 1993). Horses are sensitive to airborne particles and a strong association has been demonstrated between airborne pollution and respiratory diseases in horses (Christley *et al.* 2000; Clarke & Madelin 1987). Poorly managed horse stables with high airborne particle concentrations may affect the animals' respiratory health as well as the health of stable workers (Gruys *et al.* 1994). Horses in countries with colder climate are routinely stabled for a large part of the day so the maintenance of acceptable air quality becomes an important aspect of good stable management (Mathews & Arndt 2003). In addition, horses are kept in buildings for extended periods over many years and thus the length of exposure to airborne pollutants is significantly greater than for food animals (Clarke & Madelin 1987; Vandenput *et al.* 1998). Therefore, appropriate airborne particle reduction methods have to be an integral part of routine stable management (Clarke & Madelin 1987; Dunlea & Dodd 1997). To facilitate the wider adoption of particle reduction techniques, a series of experiments have been conducted in South Australia to evaluate the effects of different

management strategies aimed at reducing airborne particle and other airborne pollutant concentrations in horse stables.

## MATERIAL AND METHODS

The specific objective of the study was to assess the effects of three different bedding treatments on the resultant air quality using controlled experiments. The effectiveness of these treatments was evaluated by comparing the air quality parameters with corresponding data obtained in a “standard” sawdust bedded stable (control). The effects of (1) sawdust impregnated with canola oil at the inclusion rate of approximately 7% (weight/weight), (2) straw bedding and (3) “horse-nappies” (that prevents the bedding material to be contaminated with faecal material) on the concentration of airborne particles inside four horse stables were studied, using 4x4 Latin Square experimental design over four weeks. The advantage of the Latin Square design is that it effectively controls for different sources of variation that may possibly increase experimental errors (Chen & Chen 1999; Demidenko & Stukel 2002; Tukey 1997). The boxes were cleaned between experiments to avoid any carry over effects from previous treatments. Each box received the treatment for a week and then the treatments were re-allocated randomly.

**Table 1.** Experimental design for the horse trial

Week	Horse box A	Horse box B	Horse box C	Horse box D
1	Straw treatment*	Control	Oil treatment	Nappy treatment
2	Oil treatment	Nappy treatment	Straw treatment	Control
3	Control	Straw treatment	Nappy treatment	Oil treatment
4	Nappy treatment	Oil treatment	Control	Straw treatment

\*Straw treatment: straw bedding, without nappy and without oil spraying; Oil treatment: saw-dust bedding, without nappy and with oil spraying; Control: saw-dust bedding, without nappy and without oil spraying; Nappy treatment: saw-dust bedding, with nappy and without oil spraying

Air quality parameters were recorded for 28 days in the four naturally ventilated horse boxes housing one horse each. Airborne inhalable and respirable particles, carbon dioxide, humidity and temperature were measured as previously described in detail by Banhazi et al (2004). In brief, temperature and humidity data were recorded using Tinytalk temperature and humidity loggers (Hasting Dataloggers, Tinytalk-2). Total inhalable and respirable particle concentrations were measured using air pumps connected to cyclone filter heads (for respirable particles) and Seven Hole Sampler (SHS) filter heads (for inhalable dust) were operated at 1.9 and 2.0 l/min flow rate, respectively. Dust pumps were operated from 09.00 to 15.00 hours. Carbon dioxide was monitored using Multi-gas Monitoring Machines to confirm that ventilation rates were similar in the boxes and to measure ammonia levels (Banhazi *et al.* 2005). The air quality data was analysed using GLM procedure in SAS and parameters were compared between the treatments.

## RESULTS AND DISCUSSION

Temperature and the concentration of carbon dioxide did not vary significantly throughout the experiment but there was a statistically significant ( $p=0.006$ ) reduction in the concentration of

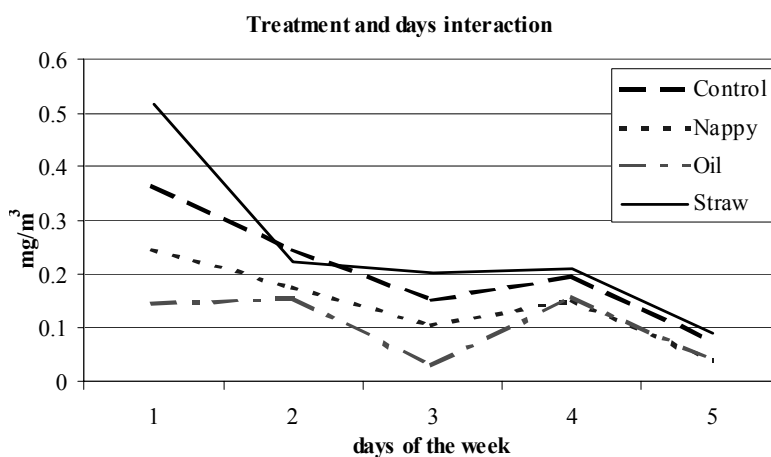
inhalable particles (Table 2). On average, inhalable particle concentrations were the highest for the “Straw” followed by the “Control” treatments. The “Nappy” and “Oil” treatments gave the lowest concentrations of inhalable particles and the difference between these two treatments was not significant.

**Table 2.** Temperature and the concentrations of inhalable airborne particles and carbon dioxide for the control and treatment boxes.

Treatment	Temperature (°C)	Inhalable dust (mg/m <sup>3</sup> )	Carbon dioxide (ppm)
Control (saw dust)	22.2 <sup>a</sup>	0.397 <sup>a</sup>	499 <sup>a</sup>
Straw bedding	22.5 <sup>a</sup>	0.606 <sup>b</sup>	488 <sup>a</sup>
Horse-nappy	22.2 <sup>a</sup>	0.287 <sup>c</sup>	508 <sup>a</sup>
Oil-impregnated saw dust	22.3 <sup>a</sup>	0.298 <sup>c</sup>	504 <sup>a</sup>

<sup>ab</sup> Values in the same column with different superscripts differ significantly ( $P < 0.05$ ).

Respirable particle concentrations were also positively affected by the treatment, but only in interaction with the “Day” effects (Figure 1).



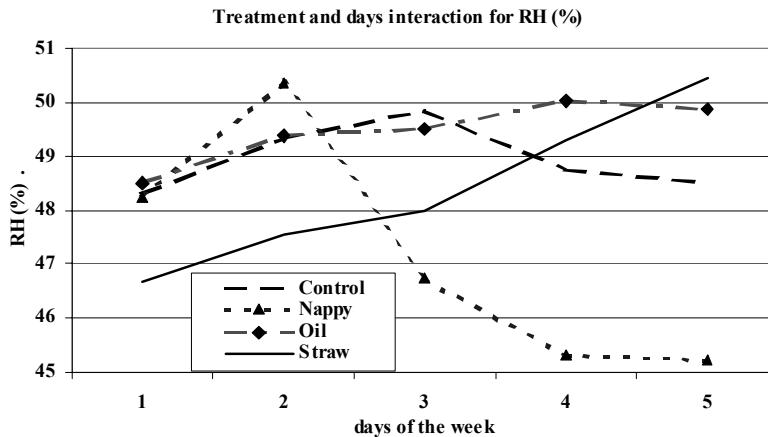
**Figure 1.** The effects of treatment and day interaction on respirable particle concentrations (mg/m<sup>3</sup>)

The interaction was mainly influenced by the readings from the first day of the weekly measurements. On the first day of the week (Monday) there was considerable variation between the treatments with the “Straw” treatment having the highest readings of respirable dust (Figure 1). This variability or difference between treatments decreased over the subsequent measurement days. However, as an overall trend it can be seen from the graph that the highest concentrations of respirable particles were recorded for the “Straw” and “Control” treatments compared with “Nappy” and “Oil” treatments. The “Oil” treatment gave the lowest concentrations of respirable particles, compared to all other treatments (Figure 1).

Differences between relative humidity readings were also highly significant ( $p < 0.001$ ), indicating that adjusting for this co-variate within the analysis was important (Figure 2).



Treatment and Day effects significantly interacted ( $p=0.041$ ) for this variable (Figure 2). Relative humidity on average increased over the 5-day measurement period for “Oil” and “Control” treatments, but decreased for the “Nappy” treatment over the experimental days. This is likely to be due to the horse nappy preventing contamination of the bedding material with faecal matter.



**Figure 2.** The effects of treatment and day interaction on relative humidity (RH, %)

These results demonstrate a significant reduction in the concentrations of inhalable and respirable airborne particles in horse boxes using either oil-impregnated bedding material or horse nappies can be achieved. These techniques would enable horse keepers to improve the environmental quality of horse stables at a relatively low cost. However, further studies are needed to determine the best method of incorporating oil into the bedding material, the minimum concentration of oil necessary and the effects of oily bedding material on the health and wellbeing of the animals.

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## A LABORATORY STUDY OF CLEANABILITY OF SURFACES FOR USE IN PIGGERIES

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### SUMMARY

The aim of this laboratory study was to examine the effects of plastic coatings on cleanability of concrete flooring for use in piggeries. According to both colorimetric and radiochemical measurements, coating of concrete improved the cleanability of floorings intended for use in piggeries. The quantitative radiochemical method appeared to be an excellent way to detect soil absorbed in materials. This is important especially from the point of view of surface hygiene in animal houses. A colorimeter detects soil visible on surfaces, gives results instantly and can be used both in practical conditions in animal houses and in laboratory studies.

**Keywords:** animal house, piggery, flooring, cleanability, radiochemistry, colorimetry

### INTRODUCTION

Material choices in animal houses are an important factor affecting the well-being of animals by allowing their species-characteristic behaviour and preventing injuries and diseases. For example the flooring should not be slippery or act as a reservoir of harmful microbes. Material choices also affect the comfort and safety of the personnel working in animal production buildings. When the aim is to produce safe food products, requirements for hygienic properties of the production plants are also of importance. Furthermore, the hygienic environment of the animals affects the quality of meat. Both chemical substances and mechanical impact on floorings cause corrosion and wearing that may promote injuries to the animals. In addition they may make cleaning difficult, thus promoting spreading of diseases (DeBelie, 1997). Therefore the use of coatings to protect the surface of concrete against wear is of interest. For example polyurethane has sometimes been used in cow houses and horse stalls, but its use in animal floorings is not widespread. The aim of the study was to examine the effects of plastic coatings on cleanability of concrete flooring for use in piggeries.

## MATERIALS AND METHODS

Concrete, the most common material for solid and slatted floors of piggeries, and five plastic coatings were examined (Table 1). All materials and methods were presented in detail in Kymäläinen et al. (2007). The surfaces were examined both as new and as worn. Wearing was carried out by grinding the surface of the tiles for with a floor grinding machine ( $t = 30$  s,  $m = 37.6$  kg, sandpaper disc n:o 60  $\phi = 400$  mm,  $p = 0.3$  N/cm<sup>2</sup> = 3.0 kPa). The surface materials examined were characterized with SEM (scanning electron microscopy) and a laser profilometer.

**Table 1.** Codes and types of examined surface materials. All coatings were based on concrete tiles

Code	Type
EP mass	Epoxy mass including sand, finished with rubbing
EP concrete mass	Epoxy + cement mass, sand scattered on the top
MDI PUR	MDI-based polyurethane, sand scattered on the top
PUR concrete mass	Polyurethane + cement mass, sand scattered on the top
Rubber PUR	Rubber-polyurethane, rubber crush scattered on the top
Concrete	Concrete

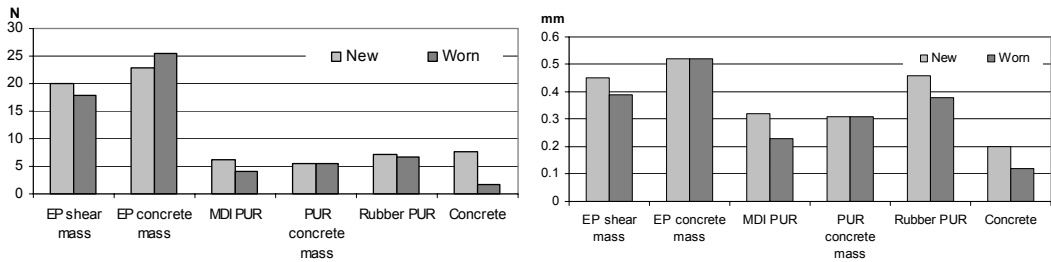
The natural manure soil and other model soils were used for the cleanability experiments (Table 2). Radiochemical and colorimetric methods were used for assessing the cleanability result. The cleaning system for the colorimetric study consisted of a pressure cleaner (120 Bar = 12 MPa, angle of the nozzle 45° to perpendicular, T of water = 10°C, 40°C, 75°C), a conveyer belt to move the samples ( $v = 0.9$  m/s, distance between the washing nozzle and sample surface = 18 cm). The samples were subjected to three soiling and cleaning cycles at each temperature. In addition, two different radiochemical methods, a gamma spectrometric method and liquid scintillation counting, were used for evaluation of the cleanliness of the surfaces. The use of different radio isotopes enables the determination of different components of soil. <sup>51</sup>Cr labels particle components and <sup>14</sup>C oil components of the model soils.

**Table 2.** Model soils used in the cleanability study and details of soiling

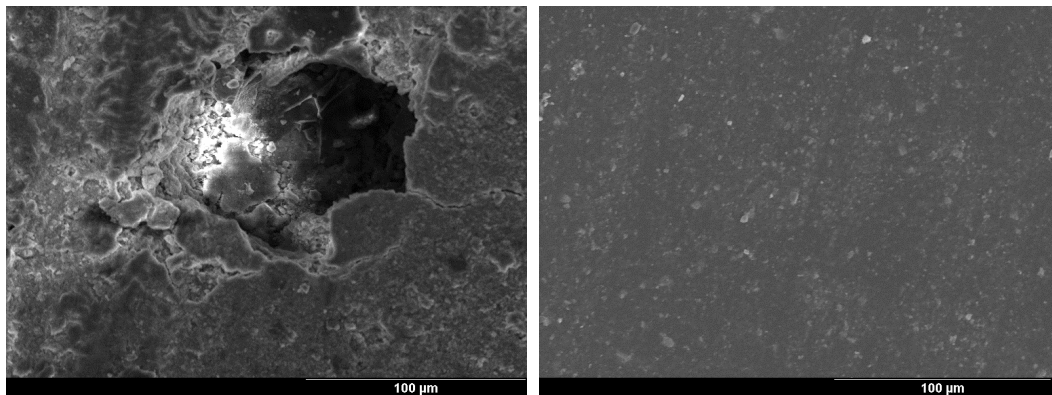
Method	Model soil	Amount of soil per sample,	Drying time after soiling, d
Colorimetric	Paste model soil (Puumala and Lehtiniemi 1993) containing paste, rye flour, sawdust, water and red caramel color	38 ml	7
Colorimetric	Manure soil: pig sludge manure containing sawdust	45 ml	14
Radiochemical	Inorganic particle and oil soil: Cr <sub>2</sub> O <sub>3</sub> , C <sub>57</sub> H <sub>104</sub> O <sub>6</sub> , <sup>51</sup> Cr	50 µl	1
Radiochemical	Inorganic particle and oil soil: Cr <sub>2</sub> O <sub>3</sub> , C <sub>57</sub> H <sub>104</sub> O <sub>6</sub> , <sup>14</sup> C	50 µl	1

## RESULTS AND DISCUSSION

As can be seen in Figure 1, in general the effect of wearing on the surface roughness was minimal. Although the surface of concrete was visibly porous, the roughness values selected did not indicate any difference between the pores and peaks of the surfaces. The porosity of concrete was more clearly illustrated by SEM figures (Figure 2).

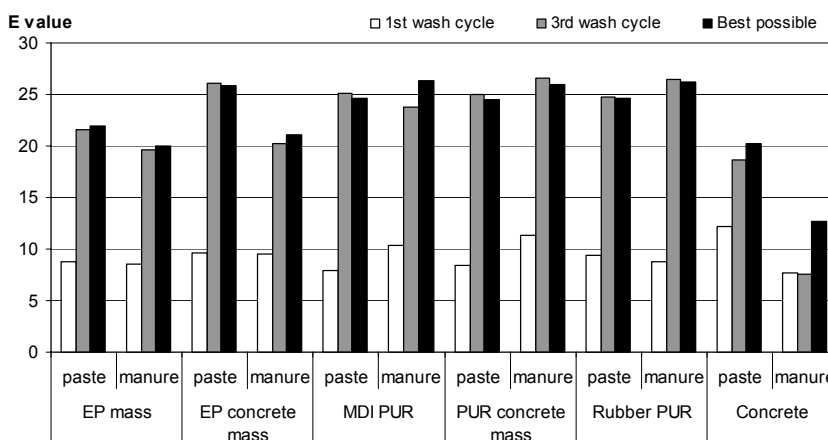


**Figure 1.** Mean roughness of new and worn surface materials as high spot count per 100 mm (left) and as peak average height (mm, from the mean centre line, right), determined with a laser profilometer. Results are means of 5 replicates with three independent measurements per replicate



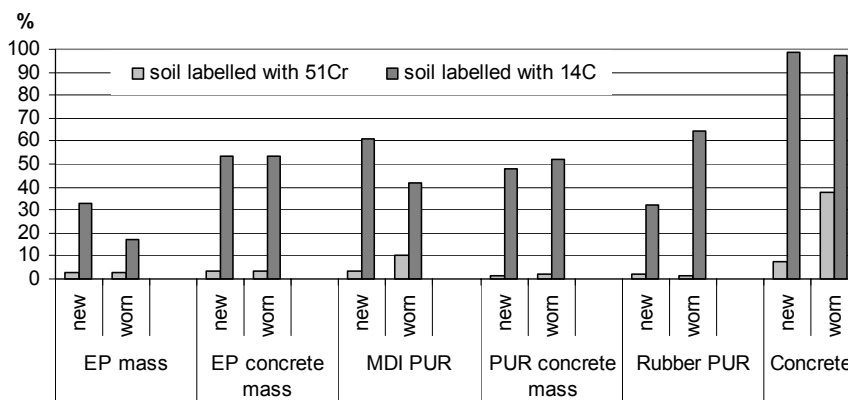
**Figure 2.** Examples of the SEM micrographs of new concrete (left) and rubber polyurethane coating (right), magnification x500

According to the colorimetric measurement the three different washing temperatures did not affect the cleanability of the surfaces from the paste soil. Therefore only the results of the warm wash (40 °C) are presented in Figure 3. The coatings increased the cleanability of concrete. Uncoated concrete had the poorest cleanability. In the case of all surface materials both soils were mainly only wetted during the first wash cycle and removed during the second cycle. The third cycle did not improve the cleanability result. The effect of coating on cleanability was statistically significant but there was no significant difference between different coatings. There were only small, partly contradictory differences in cleanability of the coated surfaces from the two soils. Higher deviation in the E values of the manure soil compared to that of paste soil was mainly due to uneven removal of the manure soil.



**Figure 3.** Colorimetrically determined cleanability of worn floorings from paste and manure soils using warm water (40°C) in the three washing cycles, compared with the best theoretically possible cleaning results. In order to define the best possible E value, theoretical E values were calculated using colorimetric values of the unsoiled surfaces. Results are means of six replicates. The higher the E value, the better is the cleanability result.

As can be seen in Figure 4, the radiochemical model soil with <sup>51</sup>Cr was removed more efficiently from surfaces than the soil labelled with <sup>14</sup>C. This indicates that the particle components of soils were removed more efficiently from the surfaces than the oil components. As in the case of the colorimetric measurement, coating of concrete was found to affect the cleanability, that of the uncoated concrete being the poorest. In the cases of both model soils the coatings did not differ statistically from each other. However, when individual materials were compared, more detailed information about the differences between the coatings was obtained with the radiochemical than with the colorimetric method. In addition, the difference between the coatings and uncoated concrete was more evident in the radiochemical method than in the colorimetric measurement.



**Figure 4.** Cleanability of new and worn floorings from manure soil using radiochemical measurement. Results of soil residues (%) are means of five replicates. The lower the soil residue, the better is the cleanability result.

In the case of all soils in both the colorimetric and radiochemical studies, no general differences were observed between the new and worn surfaces. However, when examining the radiochemical results, soil residues on worn surfaces of rubber PUR and concrete were greater than on the new surfaces of these materials, whereas the colorimetric method showed no difference between the new and worn surfaces. There was a significant correlation between the cleanabilities of the surfaces from both radiochemical soils (soil residue %) and manure soil.

In earlier studies concerning the cleanability of materials in animal houses (Sundahl, 1974; Hörndahl, 1995; Puumala & Lehtiniemi, 1993; Larsson, 2000; Zhang et al., 2006), visual and qualitative evaluation methods were mainly used. Other methods used are microbiological contact methods (Larsson 2000, Pelletier et al. 2002), a protein test (Larsson 2000) and optical methods (Zhang et al. 2006). In the present study both the semi-quantitative colorimetric method with real manure and artificial paste soils, and the quantitative radiochemical method with simplified model soils gave similar results concerning cleanability of the examined surfaces. This is a very important methodological result for further studies. However, in the present study all other examined surfaces were non-porous except for the uncoated concrete. In the case of porous surfaces the radiochemical method is of even greater importance, since it also detects soil from inside the material.

Ideal flooring is a compromise (Baxter 1984) or a balance between different properties (Shaw 1988). Cleanability is one important factor determining the hygienic properties of the floorings, which in turn affects both the well-being and health of the pigs and the hygienic quality of products. However, coatings increase the cost of the flooring and therefore the coating might be used at selected sites of high hygienic importance in piggeries. Furthermore, preventing slipperiness should be taken into consideration.

## CONCLUSIONS

According to both colorimetric and radiochemical measurements, coating of concrete improved the cleanability of floorings intended for use in piggeries. The use of coatings can thus be justified in sites in which a high level of cleanliness is required, probably meaning that coatings will be used only in some sites in animal production buildings. According to the colorimetric results, coating also decreases the time required for cleaning. Cleanability should be taken into consideration when selecting materials for sites with hygienic requirements. From the methodological point of view, in this study we demonstrated the applicability of two different evaluation methods for soiling and cleanability of agricultural surfaces, namely the radiochemical method for laboratory studies and colorimetry for both laboratory studies and field use.

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## STUDIES ON ALTERNATIVE TEST PARAMETERS FOR VIRUCIDAL TESTING OF CHEMICAL DISINFECTANTS ACCORDING TO THE GUIDELINES OF THE GERMAN VETERINARY ASSOCIATION (DVG)

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### SUMMARY

The testing of chemical disinfectants in animal husbandry and food hygiene is an important contribution to control the spread of epidemic diseases and to ensure consumer protection. In Germany testing is carried out according to the guidelines of the German Veterinary Society (Deutsche Veterinärmedizinische Gesellschaft e.V., DVG). Virucidal testing is only required for animal husbandry, not yet in food hygiene. The current DVG guidelines are not in accordance with the European guidelines (CEN) which among other differences require testing at temperatures of 10° and 20°C.

The aim of the study was to examine in suspension tests whether the test temperature has a significant influence on virus inactivation. The existing guideline also recommends assessing the impact of a defined amount of protein on virus inactivation. Therefore, the influence of protein contamination was compared to the impact of a decreased testing temperature. By analysing five commercial disinfectants and eight viruses [vaccine virus, Newcastle disease virus (NDV), reovirus, ECBO virus, ECHO virus, feline panleukopenia virus (FPV), feline calicivirus (FCV) and bovine viral diarrhoea virus (BVDV)] the impact of the protein contamination had a similar impact as the temperature (62% versus 42% of the samples tested differently at the respective settings).

Another issue that was addressed in this study was to examine other viruses as potential model viruses for disinfectant testing. The currently used viruses (ECBO virus, reovirus, NDV, and vaccine virus) were compared to ECHO virus and FPV, as potential alternative viruses for ECBO virus, or compared to BVDV and feline herpesvirus as potential alternatives to NDV, or compared to FCV as a potential alternative to reovirus. The main criterion was resistance to five commercially available disinfectants based on the main classes of chemical disinfectants, i.e., peracetic acid, aldehydes, Quats, organic acid.

ECHO virus was shown to be more resistant than ECBO virus or FPV, and BVDV was more resistant than NDV and FHV. As BVDV is much easier to handle, this virus would be a very interesting alternative to replace NDV which needs to be propagated in eggs. FCV was found to display a very similar tenacity than reovirus and may be considered as a valuable test virus once virucidal testing is implemented also for testing disinfectants for use in food hygiene.

## COMPARISON OF TWO SURFACE TEST METHODS, EN 14349 AND OECD, FOR THE EVALUATION OF THE BACTERICIDAL ACTIVITY OF DISINFECTANTS FOR VETERINARY PURPOSES

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### SUMMARY

The aim of this study was to compare the surface test method EN 14349 – “Quantitative surface test on non-porous surfaces without mechanical action” with the “Guidelines for evaluating microbicides on surfaces” discussed at the OECD. Both methods were evaluated with regard to their effects on the test results and on the recommendations for practical use.

The results obtained with the test organisms *Staphylococcus aureus* and *Pseudomonas aeruginosa* and the disinfectant glutaraldehyde indicate that the CEN methods are more representative for practical conditions than the proposed OECD testing. The latter results in a recommendation of too low use concentrations and exposure times.

**Keywords:** disinfectant testing, CEN, OECD, surface test, bactericidal activity, veterinary area

### OBJECTIVES

The surface test method EN 14349 – “Quantitative surface test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in the veterinary field on non-porous surfaces without mechanical action” is a harmonized standard, which shall be applied in all European countries for assessing disinfectant efficacy on surfaces.

Following an “OECD Efficacy Workshop on Certain Antimicrobial Biocides” in April 2002 in Arlington, Virginia, USA, with the final objective to identify regulatory and scientific areas where harmonization was needed, there was a discussion about developing a surface test method based on the QCT-2 test method published by Springthorpe and Sattar (2005). The objectives of this study were to compare the two surface test methods regarding the effect of the different methods on the test results and on the recommendations for practical use. The tests were performed at the Institut für Umwelt- und Tierhygiene at the University of Hohenheim.

### MATERIALS AND METHODS

EN 14349 describes a surface test method using stainless steel discs with 2 cm diameter as test surfaces. The test was carried out under simulated high level soiling conditions with a mixture of 10g/l yeast extract + 10 g/l bovine albumin serving as interfering substances.

Two min prior to the actual test 1 ml of the bacterial test suspension containing  $1.5-5 \times 10^9$  cfu/ml was added to 1 ml of the interfering substance and mixed. The test surfaces were placed in

an open Petri dish ensuring that the stainless steel discs were in horizontal position. Then they were inoculated with 0.05 ml of the test suspension and dried in an incubator at 37°C for 45–55 min “until they were visibly dry”. After drying the temperature of the surface was adjusted to room temperature. Then the inoculum was covered with 0.1 ml of the product test solution, or for the water control with water of standardized hardness instead of the product. After the chosen exposure times the surfaces were transferred into separate flasks containing 10 ml of an appropriate neutralizer and glass beads. After a neutralization time of 5 min a series of tenfold dilutions were prepared in tryptone-NaCl solution. The number of surviving test organisms was determined quantitatively using the spread plate technique by spreading 1 ml of the neutralized mixture and each dilution step on an appropriate number of surface dried Tryptone Soy Agar (TSA) plates. The viable counts were determined after 24–48 hrs of incubation at 37°C. In parallel tests for validation of the dilution neutralization and water control were carried out. For each test organism, product test concentration, and exposure time, the reduction in viability in comparison to the water control was calculated.

The draft surface test method discussed on OECD level is not yet described in detail. Based on the QCT-2 test method stainless steel discs 1 cm in diameter serve as test surfaces and the test is performed in a closed system. For preparation of the inoculum 340 µl of the bacterial test suspension containing  $> 10^9$  cfu/ml (*Staphylococcus aureus*) or  $> 10^8$  cfu/ml (*Pseudomonas aeruginosa*), 25 µl bovine albumin, 100 µl mucin, and 35 µl of a tryptone stock solution were mixed. The stainless steel discs were inoculated with 10 µl of this test suspension dried by keeping them for two hours at room temperature in a desiccator under vacuum. The dried discs were picked up and placed on the inside bottom surface of a vial. The inoculum on each test carrier was covered with 50 µl of the product, the vials were closed and kept at room temperature for the chosen exposure times. For the water controls 50 µl of sterile saline were pipetted onto the test surface instead of the product. At the end of the chosen contact times 9.95 ml of sterile saline – T (0.85% NaCl + 0.1% Tween 80<sup>®</sup>) were added to each vial and mixed for 45–60 s to recover surviving test organisms. For the determination of the viable counts the content of each vial and the rinsing liquid of 3 washing steps with a total of approximately 40 ml saline were filtrated in a membrane filtration equipment using membranes with a pore size of 0.45 µm. In parallel spread plate technique was used for the determination of viable counts. All plates were incubated at the 37°C for 48 hours.

For each test organism, product test concentration, and exposure time the reduction in viability in comparison to the water control was calculated.

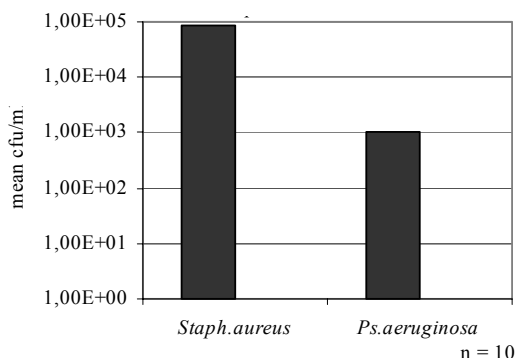
*Staphylococcus aureus* and *Pseudomonas aeruginosa* were used as test organisms, and glutaraldehyde with exposure times of 5, 30 and 60 min was used for disinfection.

The product was deemed to have passed the surface test if it demonstrated a 4 lg reduction within the chosen contact times at 20°C or room temperature.

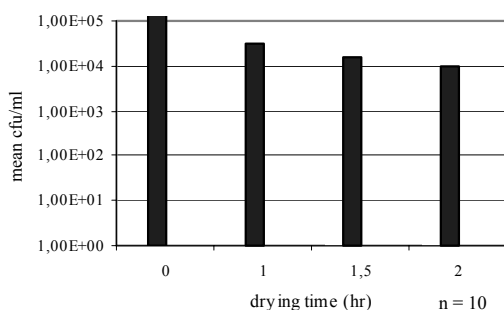
## RESULTS

If *Pseudomonas aeruginosa* was used as test organism according the OECD draft after drying the discs for 2 hrs in the desiccator at room temperature under vacuum, maximum numbers of  $1.89 \times 10^4$  cfu/ml were recovered from the test surfaces. So the amount of cells remaining on the test surface after drying was too low to determine the required 4 lg reduction (figure 1). Tests were

carried out to determine the loss of this gram negative test organism during drying and to fix an appropriate drying time (figure 2).

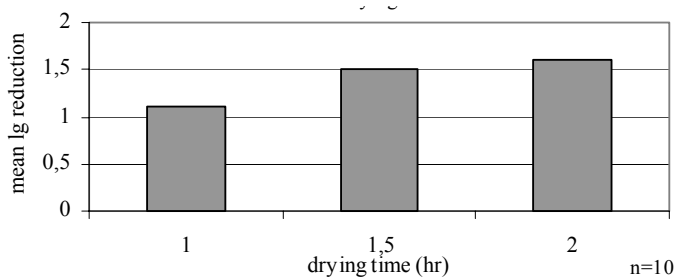


**Figure 1.** Recovery rates of the test organisms in the water control after drying the inoculated discs for 2 hrs in a desiccator at room temperature under vacuum



**Figure 2.** Mean cfu/ml of *P. aeruginosa* after different drying times in a desiccator at room temperature under vacuum

The results showed that after drying for 1 hr a loss of approximately 1.2 lg, after 1.5 hrs a mean log reduction of 1.5, and after 2 hrs drying a log reduction of 1.4 to 2.6 lg occurred (figure 3). For further testing a drying time of 1 hour at room temperature under vacuum was chosen.



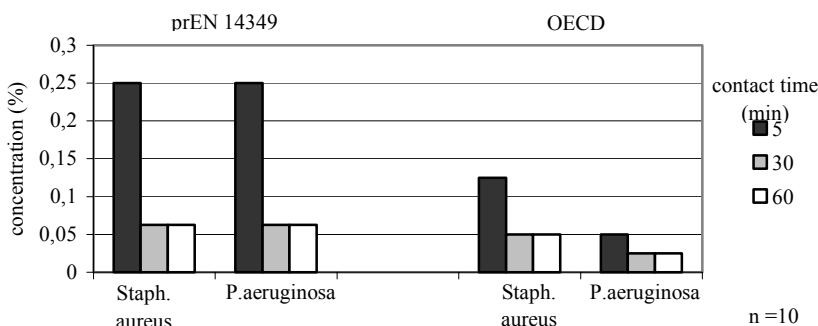
**Figure 3.** Mean lg reduction of *Pseudomonas aeruginosa* at different drying times at room temperature under vacuum

The results of trials where the different interfering substances according CEN and OECD were tested in the OECD method indicate that they are comparable and may both be used as interfering substances for high level soiling in the surface test, however the statistical evaluation is pending.

Due to the experience that membrane filtration method, which is recommended in the OECD draft for the determination of viable counts may lead to different results than when viable counts are determined using the spread plate technique, the OECD test was performed as described but determination of surviving test organisms was carried out using membrane filtration and spread plate technique in parallel. The results indicate that higher numbers of colony forming units were detected when using spread plate technique compared to the membrane filtration method. This might be due to added stress for already damaged test organisms or incomplete removal of some disinfectants during the membrane filtration procedure so that there is a remaining inhibiting effect on the growth of the test organisms.

Differences between the test results were also observed when the OECD method was performed in closed and open vials. As expected the lg reductions of the test organisms were higher when performing the test in closed vials. One explanation for these findings is that the disinfectant cannot evaporate during the exposure times and the aerosolized disinfectant still has an effect on the test organisms. Though this condition does not correspond to real life conditions.

Regarding the concentrations which passed the tests it could be shown that lower concentrations were sufficient to achieve the required 4 lg reduction in the OECD test than in the CEN test (figure 4).



**Figure 4.** Concentrations which passed the test, prEN 14349 or OECD

Final results of the statistical evaluation of the test results are pending.

## CONCLUSIONS

The analysis of the results obtained so far indicates that the CEN test methods are more representative for practical conditions than the proposed OECD testing. This may lead to big differences in the recommended concentrations and contact times depending on the method used. The latter results in a recommendation of too low use concentrations and exposure times.

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## SPECIFIC CLEANING AND DISINFECTION PROCEDURES FOR *SALMONELLA* INFECTED PIG HERDS

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### SUMMARY

The paper describes the in-depth analysis of the reasons for an extreme high *Salmonella* load of a high-health and well-managed pork production system and the specific cleaning and disinfection measures that were taken to reduce the prevalence of *Salmonella* antibody positive finisher pigs produced by the system. The results and experiences gained during the study are discussed.

**Keywords:** salmonella load, high-health pork production system, cleaning and disinfection

### BACKGROUND AND OBJECTIVE

In September 2002, the first German nation-wide quality management and assurance system for food production was launched. This QS-System (“QS” stands for “Quality and Safety”) started with the pork production chain in response to a series of scandals and a growing distrust of the consumers in meat, especially in pork. QS is a non-governmental voluntary quality management system developed and established solely by the following five sectors of the food production chain: the feed industry, farming, the slaughter industry, the meat processing industry and retail.

One of the major modules of the QS-System is a *Salmonella* monitoring programme. Due to the fact that slaughter plants and meat processors have already good hygiene procedures (GHP) and good manufacturing procedures (GMP) which include activities targeted at *Salmonella* reduction, the *Salmonella* monitoring within the QS-System focuses on the primary production, i.e. mainly on the finishing phase of the pig production. The QS *Salmonella* monitoring programme aims at categorising the participating herds according to the risk of introducing *Salmonella* into the pork chain via infected slaughter pigs. The following three categories are differentiated: Cat. I = low risk, Cat. II = medium risk, and Cat. III = high risk. The classification into the categories is calculated quarterly based on the percentage of salmonella antibody positive meat juice samples during the last 12 months for each farm (ANONYMOUS, 2007a).

The presented study is a contribution to a better understanding of *Salmonella* infection sources and reservoirs in pig production systems, especially in those with remarkably high hygiene levels, where producers and their farm veterinarians are often at a loss convinced of the idea that nothing can be improved. The objective of this study was to detect “hidden” *Salmonella* infection sources and reservoirs in a well-managed group of pig herds with a hygiene level far above average.

## MATERIAL AND METHODS

Three very cooperative owners of well-managed herds with a high hygiene level, but continuously categorised into Cat. III, were chosen for this study. All of them did not see any of the traditionally accepted risk factors (e.g. frequent diarrhoea, rodent infestation, hygiene deficiencies, pets in the barn etc.) on their farms. Furthermore, they themselves and their veterinarians did not know where to start with intervention measures.

The study herds are:

- a breeding herd with 680 sows with an extremely well-run biosecurity system (only shower-in access to the barn, separate isolation barn for gilts, ectoparasite-free status),
- a well-managed, visually always clean separate nursery (1000 piglets with 6 to 18 kg on flat decks, 1000 grow-finishers with 18 to 30–40 kg on slatted floors), and
- three finisher herds that receive exclusively weaner pigs from this breeding herd through the described nursery.

In the first phase of the study, selected and earmarked sows, piglets, weaners and finishers were repeatedly tested serologically (SALMOTYPE<sup>®</sup> Pig Screen ELISA, Labor Diagnostik Leipzig, Leipzig) and bacteriologically (DIN ISO 6579) for identifying the time and location of the infection. 42 sows were included into the study representing animals of different litter numbers ranging from sows with one litter to sows with 12 litters. All 42 sows farrowed within three weeks. Per sow three piglets were chosen, earmarked with individual numbers and blood was drawn from each of these sentinel animals at various points in time until slaughter. All together, 694 blood samples, 41 colostrum samples and 66 meat juice samples were investigated.

Simultaneously, along the first phase of the study, diverse faeces samples, environmental samples (floors, walls, fans, troughs, drinkers, transport vehicles and cleaning tools) and slaughter samples (tonsils, Lnn. mandibulares and Lnn. iliaci) were cultivated for Salmonella, all together 538 samples.

In the second phase, targeted intervention measures were implemented according to the findings of phase 1. The major measures are:

### Cleaning and disinfection (ANONYMOUS, 2007a)

- intensifying cleaning and disinfection of floors, walls, troughs, drinkers etc. and other pig contact areas in the pens
- adding disinfection to already existing cleaning of floors and walls of areas with no or rare pig contact (ante-rooms for changing clothes and boots, alleys for pig movements, tools for cleaning and devices for moving pigs, transport vehicles)
- cleaning and disinfection of areas that are not regularly included in cleaning and disinfection (fans and air ducts, upper parts of walls and ceilings, scales, loading and unloading ramps)

### Implementing “black and white” principles (ANONYMOUS, 2007a)

- optimising animal and people movement targeting for salmonella transmission
- ante-rooms with a strict and obvious separation between normal and farm clothes and boots (e.g. installing solid separation between “black” and “white”)
- installing boots use in only one building
- increasing awareness of crossing walkways between stables and farmyard



Watering system

- chlorination of the drinking water, if taken from a well (ANONYMOUS, 2007b)
- switch to municipal water supply instead of well

Changing feed structure, composition, and feed acidification (VISSCHER, 2006)

- rough grinding of grain components (largest possible particle size)
- increase of barley in the ration (about 35%)
- adding of 0.6 to 1.2% K-diformate (Formi<sup>®</sup>)

Optimising rodent control (ANONYMOUS, 2007a)

- Improving cleanliness outside barns
- Engaging a professional pest control company

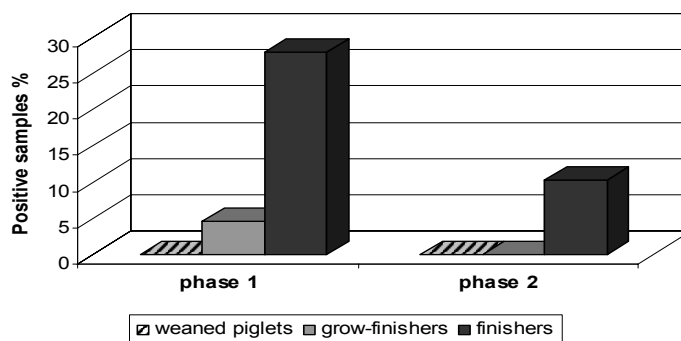
For controlling the efficacy of these measures, 357 serological samples (298 blood samples and 59 meat juice samples) were taken during phase 2. Twenty weaning pigs per finishing herd (n = 60) were randomly selected and earmarked as sentinel animals and five times serologically investigated.

Simultaneously, along the second phase of the study, diverse faeces samples, environmental samples and slaughter samples (similar as described for phase 1) were cultivated for Salmonella, all together 549 samples.

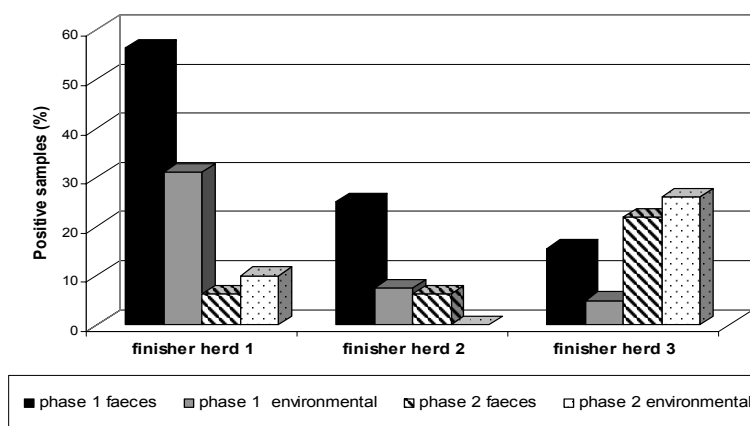
## RESULTS

Bacteriology:

1. The isolated Salmonella strains in all herds and all age groups belonged to the serovar *Salmonella Typhimurium* [4, (5), 12: i: 1, 2] and the same phage type.
2. All gilts were Salmonella negative, 8.3% of the pooled faeces samples taken from the productive sows were Salmonella positive.
3. None of the faeces samples taken from the weaned piglets in the flat deck were Salmonella positive (see Figure 1).
4. Whereas 4.5% of the grow-finisher samples in phase 1 were Salmonella positive, none of these faeces samples were Salmonella positive in phase 2 (see Figure 1).
5. The drastic increase of Salmonella positive faeces samples from grow-finishers to the finishers in phase 1 from 4.5% to 27.8% was remarkably reduced in phase 2 to 10.2% in the finishers (see Figure 1).
6. The bacteriological results of samples (faeces and environmental) taken from the finisher herds 1, 2 and 3 show only in herds 1 and 2 significant reductions between phase 1 and phase 2, whereas in herd 3 an increase occurred (see Figure 2):
  - herd 1, phase 1: faeces 56.3%, environmental 31.1%
  - herd 1, phase 2: faeces 6.3%, environmental 10.0%
  - herd 2, phase 1: faeces 25.0%, environmental 7.5%
  - herd 2, phase 2: faeces 6.3%, environmental 0%
  - herd 3, phase 1: faeces 15.6%, environmental 5.0%
  - herd 3, phase 2: faeces 21.9%, environmental 26.1%



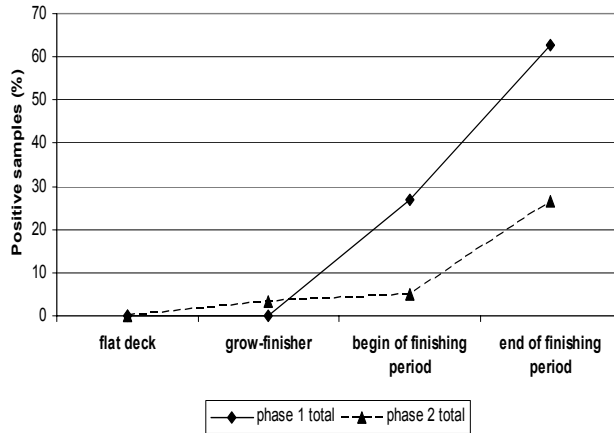
**Figure 1.** Bacteriological results of faeces samples of all three finisher herds in phase 1 and 2



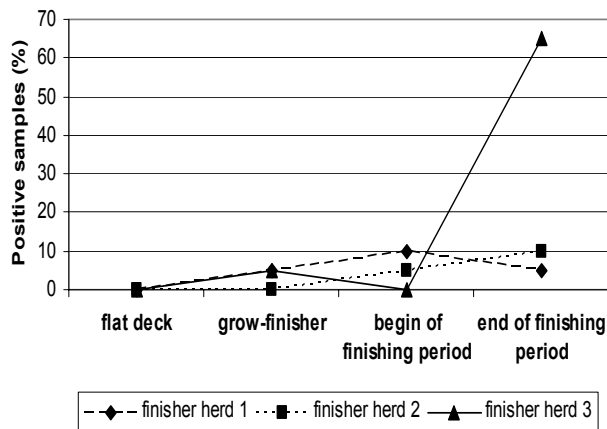
**Figure 2.** Bacteriological results of faeces and environmental samples in finisher herds 1, 2 and 3 in phase 1 and 2

### Serology:

1. The serological results of the blood samples of the sows, of their colostrum, and the blood samples of the corresponding 7-day piglets correlated highly significantly.
2. The colostral antibodies in piglets decreased drastically during the suckling period; even piglets with the highest antibody level were negative after weaning.
3. The percentage of Salmonella antibody positive samples of all three herds in phase 1 increased over time and exceeded the 40%-threshold (category III) in the end of the finishing period, whereas the overall percentage of the positive samples in the end of the finishing period of phase 2 remained below 40% (see Figure 3).
4. The reduction of the overall percentage of the Salmonella antibody positive samples in phase 2 is exclusively due to the remarkable decrease of positive samples in herd 1 and 2 (see Figure 4).
5. The reduction (herds 1 and 2) and non-reduction (herd 3) of the serological results correlated strongly with the reduction (herds 1 and 2) and non-reduction (herd 3) of the bacteriological results in faeces samples (see Figure 2 and 4).



**Figure 3.** Serological results of blood samples of all three herds in phase 1 and 2



**Figure 4.** Serological results of blood samples of herd 1, 2 and 3 in phase 2

## DISCUSSION AND CONCLUSIONS

As for its *Salmonella* infection pattern before any intervention measures, the investigated three-site pork production system (one sow herd, one flat deck with grow-finishers, and three finisher herds) can be characterised as follows:

- The “*Salmonella* problem” of the production system is obviously not a constant introduction of *Salmonella* into the system at various points of entry, but rather the circulation of one “quasi” hospitalised *Salmonella* serovar.
- This serovar is already found in the sow herd, but the *Salmonella* prevalence of the weaned piglets in the flat deck and in grow-finishers on the same site as the flat deck is relatively low.

- This low prevalence in the flat deck and grow-finisher period, however, leads to a varying increase of the Salmonella infection rate in the three finisher herds, with remarkable differences in the resulting prevalence in the end of the finishing period.

The intervention measures taken on flat deck and grow-finisher site as well as in the three finisher herds (specific measures on each site according to the results of the in-depth analysis of phase 1 as described in material and methods) are capable of drastically reducing the infection pressure and environmental contamination in Salmonella infected pork production systems (herds 1 and 2). However, it is unrealistic to expect a complete “sanitation” during one production cycle – only the stringent repetition of the specific measures necessary to be defined for every herd can lead to a sustainable success. Any failure in reducing the Salmonella load (as in finisher herd 3) must result in another in-depth analysis of the hygiene, biosecurity and the daily working procedures on the farm in question. Such analysis will identify the reasons for the failure, if “everything that happens” on the farm is taken into consideration; in case of herd 3 a non-planned construction in the barn without biosecurity measures, and a transfer of liquid manure from a cattle shed and a pig barn to the deep pit of the study pig barn led to severe hygiene and biosecurity break-downs.

Although the study design (applying all possible measures for reducing the salmonella load at once for demonstrating the feasibility of the reduction) did not allow an exact calculation of the contribution of the cleaning and disinfection procedures to the success in herd 1 and 2, it can be said that:

- visually clean and disinfected areas need to be tested for their freedom from Salmonella, and the cleaning and disinfection procedure must be improved, if Salmonella is still found,
- most important for the Salmonella reduction (in contrast to animal-adapted, pig-specific pathogens) is the inclusion of areas and rooms, to which the animals have no direct contacts:
  - floors of hallways inside and outside the barns (hallways for moving pigs need additional cleaning and disinfection of the walls as well!),
  - ante-rooms for changing boots and clothes (regular clothes and shoes must be clearly separated from coveralls and boots that are only used inside the barn),
  - rooms for feed storage, especially around the silo outlets,
  - offices and any other rooms that are walked into with the “inside-the-barn-boots”,
- devices such as fans and areas such as under the trough and feeder outlets that are not regularly cleaned and disinfected, need to be included in the thorough cleaning and disinfection procedure in case of a high Salmonella load,
- any tools such as brooms, tools for scratching faeces, boards for moving pigs and transport vehicles need also be cleaned and disinfected.

These cleaning and disinfection procedures need to be applied (at least at the beginning of the sanitation attempt) after every movement of any pig group. In herds with a very high Salmonella load, these thorough and “more-than-usual” cleaning and disinfection measures need to be carried out and tested for their efficacy in several consecutive production cycles. The very basic precondition for the success of any sanitation, however, is that the farmer together with his veterinarian does not only follow a check list of measures, but analyses any routine activity on the farm and in the barn that might support the introduction and spread of Salmonella into the pork production system.

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## DEGRADATION OF DORAMECTIN DURING THERMOPHILE PHASE OF COMPOSTING AND MANURE STORAGE

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**Keywords:** composting, doramectin, degradation, manure storage

### OBJECTIVES

Avermectins derived from soil microorganism *Streptomyces avermitilis* (Fisher 1990). Doramectin is an avermectin derivate, is an endectocide. In veterinary medicine, doramectin is used as an antiparasitic drug for extermination of numerous parasites in all species of domestic animals. Their mode of action based on strong binding on chloride channels in nerve cells of parasite, disruption of nerve impulses and its transmission (Martin, Robertson, & Wolstenholme 2002). Following animal treatment, it is excreted mostly (ab to 80%) in an active, non metabolized form irrespective on formulation, dosage and route of administration (Shoop, Mrozik, & Fisher 1995). Excretion period is usual long (several weeks), but main amount is excreted at sheep in 7 days (Hennessy & Alvinerie 2002; Stenersen 2004). The maximum concentration of 2186 ±145 ng/g dry faeces after single subcutaneous administration of 0.2 mg/kg body weight of doramectin in dry faeces was detected on day 2 (Kolar et al. 2006). Doramectin can persist in environment for longer period of time, dependent on chemical, physical and biological conditions (Eržen Kožuh Nevenka et al. 2005). Doramectin is among avermectins medicine with the greatest harmful effect in the environment, because of their specific metabolism and action on non-target organisms. Presence of avermectins in animal faeces and pasture causes killing of some adult insects, of young, barely hatched insects; it increases destruction of their larval forms and can lead to reduction in biotic diversity (Barth et al. 1993; Halley, VandenHeuvel, & Wislocki 1993; Iwasa et al. 2005; Sommer & Bibby 2002; Suarez et al. 2003; Svendsen et al. 2003). Avermectins are extremely toxic as well to water organisms in spite of poor water solubility (Tišler & Kožuh Eržen 2006). In good animal husbandry are some possibilities like composting or anaerobic digestion to influence on containing of some pollutants. Chemical properties of pollutants have strong influence during degradability process. Substances with low water solubility, a large soil/sediment adsorption coefficient and cyclic substances degraded slowly then chain and water soluble substances (Lavrance P.Wackett & Dougl Hershberger 2001). Composting as a biotechnological process is used in organic waste management and bio-remediation of contaminated organic materials and soil (Wolfgang Fritsche & Martin Hofrichter 2005). Therefore we investigated possible degradation of doramectin during composting after a single addition into compost mixture of sheep manure to minimised spreading in to the environment. As a comparison, influence of sterile compost and manure storage on doramectin degradation was assessed.

## MATERIALS AND METHODS

Degradation of doramectin followed 21 days during thermopile phase of composting and manure storage. Degradation of doramectin addition during composting of sheep faeces was studied in pilot scale (1m<sup>3</sup>) insulated vessels in the dark controlled conditions. Four different suspensions of doramectin were used for addition into compost mixtures.

**Table 1:** Composition of suspension used as addition in our study

Components	Suspension 0	Suspension 1	Suspension 2	Suspension 3
Dectomax <sup>®</sup>	0 ml	0.5 ml	1.0 ml	2.0 ml
Ethyleate 98% (Acros Organics, USA)	11.8 g	11.8 g	11.7 g	11.6 g
Purified sesame oil (for pharmaceutical use) (KEFO, Slovenia)	up to 100 ml (86.4 ml)	up to 100 ml (85.9 ml)	up to 100 ml (85.6 ml)	up to 100 ml (84.7 ml)
Achieved concentration of doramectin	0	0.05 mg/ml	0.1 mg/ml	0.2 mg/ml

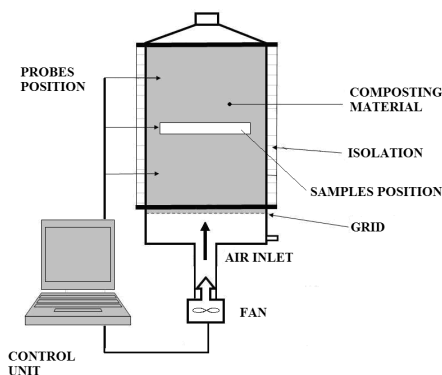
The equal quantities of each suspension were added into the compost mixtures in the single test. Target concentrations in compost were:

- Concentration 0 (C0) didn't contain any doramectin.
- Concentration 1 (C1) contend the half of concentration C2
- Concentration (C2) contend the maximum concentration detected in dry faeces after single subcutaneous administration of 0.2 mg/kg body weight of doramectin at sheep – 2186 ng/g of dry sample (Kolar, Cerkvenik Flajs, Kužner, Marc, Pogačnik, Andrej, Cornelis van Gestel, & Kožuh Eržen2006)
- Concentration 3 (C3) contend double value of concentration C2.

Each concentration of doramectin was tested in during composting in three batches (B1, B2, B3) and six samples.

**Table 2.** Sampling plan

Sampling number	Sampling day
1	0
2	7
3	14
4	21



**Figure 1.** Scheme of composting vessel.

Homogenous mixed material of sheep faeces from deep litter with addition of water and pine bark has been used so that content of moisture reached approximately 60%. Temperature was limited upward to 68°C, was controlled in vessels by PT 100 probes using computer program “Visi DaQ”<sup>®</sup> (Advantech, USA) and maintained by fans which were used for aeration of material as well. Humidity was determined by drying of samples at 105°C for 24 hours and weighing. The pH value was determined in air dried samples after addition of five amount of CaCl<sub>2</sub>, 0.01 mol/l and standing of two hours, by calibrated pH meter (Hanna HI 221, Germany). Total carbon (C), total nitrogen (N) and C: N ratio were determined by elementary analyzer Vario MAX CNS (Elementar, Hanau, Germany) by thermal conductivity detector after combustion at 900°C.

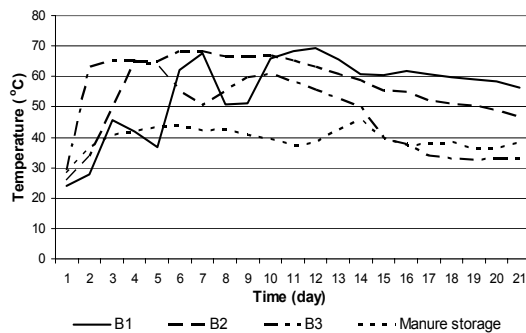
Concentrations of doramectin was analyzed using validated analytical procedure employed HPLC with fluorescent detection. Homogenized, moist samples (2.0 g) were extracted with 25 ml of acetonitrile (Merck, Darmstadt, Germany) by shaking on a mechanical shaker (Vibromix 313 EVT, Tehnica Zelezniki, Slovenia) at room temperature for 15 minutes at 400 rpm. After centrifugation for 10 min at 3000 rpm (20°C), using a centrifuge (ROTIXA/RP, Hettich, Germany), a 15 ml portion of extract was taken and mixed with 50 µl TAE and doubly distilled water to 50 ml. Bakerbond SPE Octyl (C<sub>8</sub>) cartridges 500 mg, 6 ml (J.T. Baker, Philipsburg, New Jersey) were introduced into the clean-up procedure and to pre-concentrate doramectin extracted from samples. Doramectin was eluted with 5.0 ml of acetonitrile. After that followed evaporation to dryness under nitrogen at 60°C and derivatisation. To the samples was then added 100 µl N-methylimidazole – acetonitrile (1:1, v/v) and 150 µl trifluoroacetic anhydride-acetonitrile (1:2, v/v), all supplied by Merck (Germany) (De Montigny et al., 1990), and analyzed by HPLC. The Thermo Separation Products (USA) HPLC system consisted of a Spectra Systems P2000 pump, an AS300 auto injector and a Shimadzu (Japan) RF-535 fluorescence (excitation wavelength 365 nm; emission wavelength 470 nm) detector. The separation was carried out on a Phenomenex (Phenomenex USA) Luna C18 (2) column (150 x 4.6 mm ID; 3 µm particle size) with a Phenomenex pre-column C18 (4.0 x 4.6 mm ID; 5 µm particle size). The column temperature was maintained at 28°C. Mobile phase consisting of acetonitrile, methanol (Merck, Germany) and water (47.5:47.5:6.0, v/v/v), was pumped at 1.1 ml/min and 50 µl of sample was injected into the HPLC system. Results were evaluated according to the external standard method and corrected for recovery (Kolar et al., 2004). The stock solution of doramectin in a concentration of 100 mg/ml and working standard solutions were prepared in acetonitrile. The recovery of the method was tested daily within the set of sample determinations by addition of



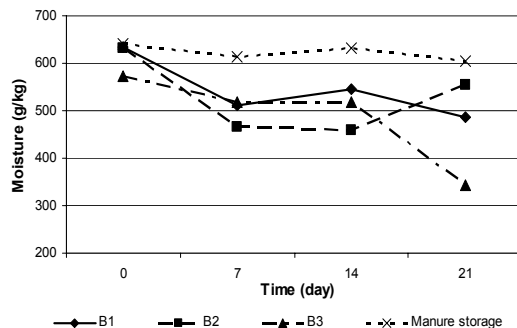
doramectin to blank moist samples at two concentrations expected in the measured samples. The blank samples (added suspension 0) served as a negative control. All samples were analyzed in four parallel determinations. Low detection limit (1.0 µg/kg of dry sample), good repeatability (RSD < 15%), recovery of the method in above 80.0%, enabled the determination of doramectin in our samples.

## RESULTS AND DISCUSSION

Average 21 days temperatures were in compost mixtures between 48.9 and 56.3°C and in manure storage 39.5°C (Figure 2). Differences in average temperatures between manure storage and all batches of composting were statistically significant ( $P < 0.05$ ). Average moisture content was in compost samples at the beginning of the study between 57.2% and 63.5% (Figure 3). Losses of the moisture were in composting mixtures between 12 and 40% and in manure storage only 5.5% (Figure 3). Losses of moisture in composting mixtures are most likely consequence of high temperatures and aeration of material (Zhu et al. 2004) in the mean time both parameters in manure storage were absent.



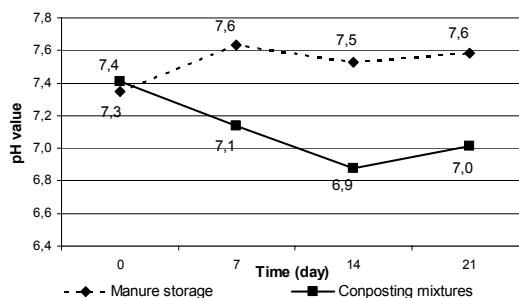
**Figure 2.** Temperature trends during composting (batches B1, B2, B3) and manure storage



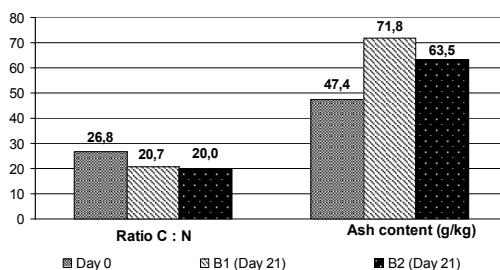
**Figure 3.** Average moisture contents during composting (batches B1, B2, B3) and manure storage.

Values of pH ranged in all samples between 6.9 and 7.66 (Figure 4). High pH values at the beginning of the study we assigned to raw material used in study (deep litter), where degradation

processes already started. Degradation products ( $\text{NH}_3$ ,  $\text{NH}_4^+$ , urea, uric acid, proteins) that originated from anaerobic degradation and can influence on pH, (Peigne & Girardin 2004); (Veecken, de Wilde, & Hamelers 2004). Values of pH decrease during composting, but in manure storage raised a little. Decrease in C: N ratio from 26:1 at the beginning of the study to 20:1 after 21 days in composting mixtures was statistically significant ( $P < 0,05$ ) (Figure 5). The tendency in C: N ratio in manure storage was opposite to composting and was grown during 21 days of storage in range 16.6: 1 at the beginning of storage up to 23.4 in day 21. Increase in inorganic matter – ash contain, decrease in C:N ratio and changes of temperatures in composting mixtures were in our study indicators of good composting process (Nakasaki et al. 1992).



**Figure 4.** Average pH values during composting and manure storage



**Figure 5.** Average ratio C: N and average ash contain on day 0 and day 21 in composting mixtures studied; B3 was not analyzed.

Degradation of doramectin during composting was also found and was in average 36. 6% (Figure 6). Differences in content of doramectin in samples before composting and day 7, 14 and 21 were statistically significant ( $P < 0,05$ ). Differences in degradation rate between varied concentrations were insignificant. Degradation of doramectin in manure storage was in average 12.2% and difference in doramectin contains in samples before storage and day 14 and 21 were statistically significant ( $P < 0,05$ ) (Figure 7).

Statistically evaluation of composting parameters showed on joining doramectin degradation and loss of moisture in samples especial in B3 ( $r = 0,969$ ) and correlation was statistical significant ( $P < 0,05$ ). About influence of moisture was published in other studies (Kolar & Kožuh Eržen 2007). We believe that decreases in doramectin concentration could not be significant dependent with aeration of material nor evaporation account of strong tendency to bind to particles and low water solubility (Bloom & Matheson 1993). During composting process formed different substances and as well humic substances (humic acids, fulvic acids, humins) (Miikki et al. 1994;

Mondini et al. 199; Huang et al. 2006). Known are sorption properties organic and inorganic molecules and also pesticides and herbicides (Bollag, Myers, & Minard 1992; Flidner 1997; Jones & Bryan 1998).

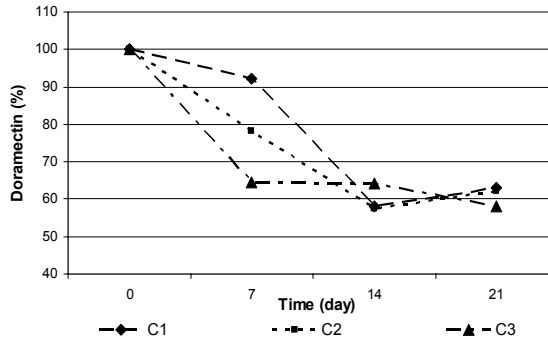


Figure 6. Average doramectin contain during composting.

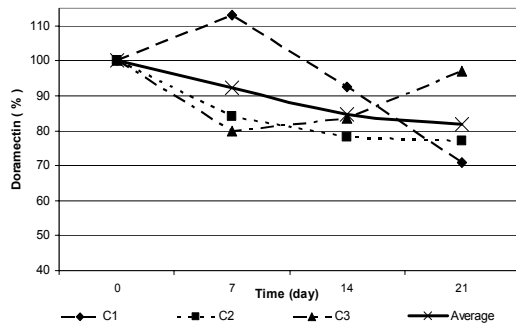


Figure 7. Average doramectin contain during manure storage.

## CONCLUSIONS

Rapid rising of temperatures in composting mixtures was proved by biodegradation of composting material. Loss of the humidity in compost mixtures result from aeration of composting mixtures. We observed gradual degradation of doramectin under composting and storage conditions. Degradation rate in 21 days was greater during composting then manure storage. This difference is due to more intensively biological degradation and loss of humidity which can influence on sorption behaviour of doramectin against organic carbon in dry matter. For final estimation, these influences should be assessed further. Faster degradation during composting could be turned into account to reduce enter of medicine in to environment.

## ACKNOWLEDGEMENTS

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## POSTER PRESENTATIONS

### INNOVATIVE SOLUTION IN MASTITIS PREVENTION THROUGH TEAT DIPPING

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#### ABSTRACT

The new teat dip formulation based on lactic acid has demonstrated bactericidal efficacy according to in-vitro European and AOAC norms against environmental and mastitis inducing germs. The action is powerful (25% dilution in EN test) and quick (1 minute contact time in AOAC). Field trials carried out in this study confirm the teat conditioning properties of the teat dip and ability to keep reduce the low level of cell count in milk. More than Iodine and Chlorhexidine products, the innovative solution for teat dipping is able to produce fly repellent and sun screen activity both suitable in summer.

**Keywords:** teat dip, lactic acid, fly repellent, bactericidal, European norms, AOAC norms

#### INTRODUCTION

Major products on the teat dipping market are based on Iodine, Chlorhexidine and Chlorine derivates. There always has been a dilemma in teat dip formulations between bactericidal activity and product toxicity in terms of cytotoxicity or environmental behaviour. Thus pushing formulators to develop low iodine technologies with high emollient contents. Lastly some Nordic countries with strategy environmental friendly policies have decided to classify biocidal products including teat dips according to Environmental behaviour, including biodegradation and bioaccumulation properties of the active substances.

Formulators therefore decided to develop alternative teat dips based on compounds such Nisin, Lactic acid, DDBSA,... The need of the farmer in additional features in teat dips makes the formulations more complex. Making the teat dip sun protective and fly repellent is challenging when the need is also to get enough the bactericidal activity. It has been notified therefore that alternative formulations such lactic acid demonstrates low efficacy in terms of mastitis prevention. Our works focused on activating the bactericidal power of lactic acid through synergistic combinations while increasing the features of the teat dip with demonstrated efficiency in terms of sun protection and fly repellent effect.

## EXPERIMENTAL

The innovative teat dip is based on a synergistic mixture of lactic acid, surfactant, fly repellent and sunscreen compounds.

The formulation has been investigated on top of the chemical and physical properties for the following experiments:

- In-Vitro testing according to European Norms on antiseptics and disinfectants EN 1656 (Determination of bactericidal activity according to standard EN 1656, chemical disinfectants and antiseptics used in veterinary field).
- In-Vitro Testing according to AOAC Official Method 960.09.
- Teat Conditioning trial and mastitis prevention, Belgian trial according to VICH GL9 guidelines for GCP and
- Fly repellence effect against *Musca domestica* (Test chambers according to AFNOR, BSI and CEB)
- Sunscreen effect expressed as absorbance versus wave length.

## RESULTS

1) In vitro testing according to European norms

**Table 1.** Results of *in vitro* testing according to EN 1656

Bactericidal tests	Effective Dilution	Reduction
<b><i>Pseudomonas aeruginosa</i></b>	25%	5 Log
<b><i>Staphylococcus aureus</i></b>	25%	5 Log
<i>Escherichia coli</i>	25%	5 Log
<i>Streptococcus agalactiae</i>	25%	5 Log
<i>Streptococcus dysgalactiae</i>	25%	5 Log
<i>Streptococcus uberis</i>	25%	5 Log

According to the results of the microbiology testing on EN norms 1656, presented in Table 1, the new teat dip formulation has a bactericidal action against mastitis causing bacteria as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus agalactiae*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis* at 25% against mastitis causing bacteria at 5 minutes of contact time. The formulation is therefore 4 times more efficient than the required level by EN testing. This efficacy is comparable to classical iodine or chlorhexidine products.

2) *In vitro* testing according to AOAC

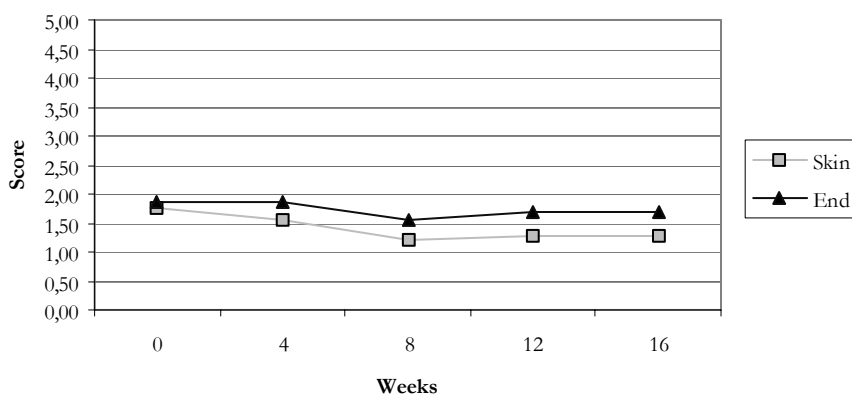
The lactic acid formulation was tested according to AOAC method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectant, the efficacy was demonstrated against the following germs:

**Table 2.** Results of in-vitro testing according to AOAC 960.09 against 8 Log CFu/ml

Bactericidal tests	Contact time	Reduction
<i>Streptococcus uberis</i>	1 minute	99,99%
<i>Staphylococcus aureus</i>	1 minute	99,99%
<i>Escherichia coli</i>	1 minute	99,99%
<i>Streptococcus agalactiae</i>	1 minute	99,99%
<i>Streptococcus disgalactiae</i>	1 minute	99,99%

The contact time being 1 minute, the innovative teat dip is demonstrating a very quick action against the mastitis inducing bacteria and in presence of milk as a challenge substance.

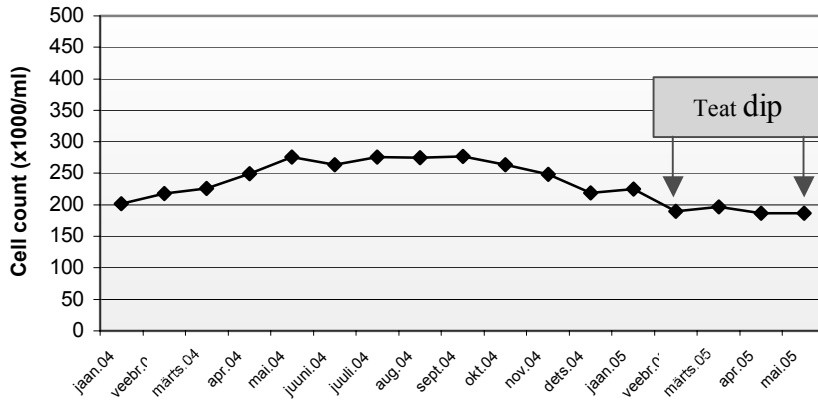
### 3) Teat conditioning trial and mastitis prevention

**Figure 1.** Evolution of teat condition during application of teat dip

The trial carried out in Belgium from 02 of February of 2005 to 24 of May of 2005 in a herd of 40 cows, demonstrates the teat conditioning properties of the lactic acid teat dip.

The scores given in Figure 1 of the teats treated with the new formulation teat dip during 16 weeks have been statistically analysed. The statistical analysis of Variance (ANOVA) allows the comparison of the mean of teat skin and end scores. This analysis of variance revealed there is significant difference between the beginning and end of the trial, the scores remain between 1 and 1,5.



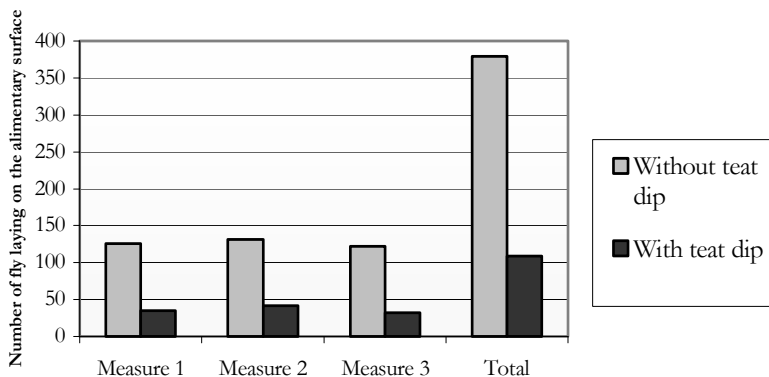


**Figure 2.** Evolution of cell count during application of teat dip

To appreciate better the evolution of the cell count during the trial and to allow statistical analysis with treatment period effect, the cell count from January 2004 to May 2005 have been recorded. Statistical comparison (ANOVA) between the results of the tested period and the same period the last year, proves that there is no significant difference between the two periods.

The new formulation teat dip is very well tolerated by cows and, on top of being strongly bactericidal, allows the improvement of a the skin condition and a low level of bulk milk cell count. These results presume the ability of the lactic acid teat dip to help prevent mastitis in dairy farms.

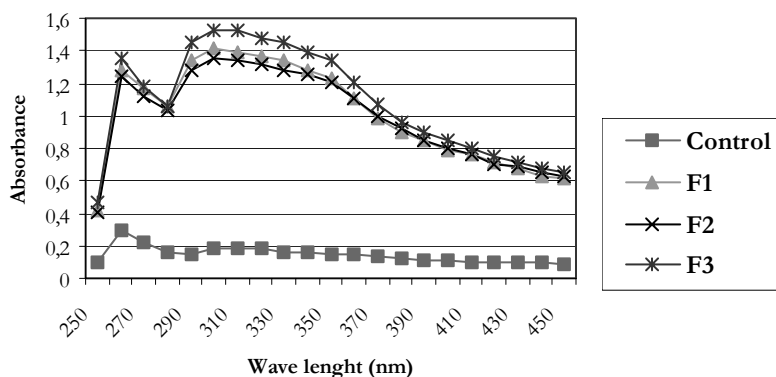
#### 4) Fly repellence



**Figure 3.** Measurement of flies laying on feeding surface treated or not with the teat dip

A laboratory validated test was carried out on *Musca domestica* to demonstrate the fly repellent activity of the lactic acid formulation. According to the results presented in figure 3, the intrinsic repellent activity of the teat dip is **71,2%**. The teat dip applied on surface demonstrates a significant repulsive effect towards *Musca domestica*. The objective of fly repellent activity of teat dip applied on teats, is reached.

## 5) Sunscreen effect



**Figure 4.** UV absorbance graph for teat dip

The sun protection factor was studied using the absorbance/SPF analysis method. Three variations of the lactic acid teat dip were tested versus a control formulation without sunscreen ingredients. All lactic acid teat dip formulations included the sunscreen demonstrated ability of sun protection.

### CONCLUSION

The new teat dip formulation based on lactic acid has demonstrated bactericidal efficacy in-vitro according to European and AOAC norms against environmental and mastitis inducing germs. The action is powerful (25% dilution in EN test) and quick (1 minute contact time in AOAC). Field trials confirm the teat conditioning properties of the teat dip and ability to keep / reduce the low level of cell count in milk. More than Iodine and Chlorhexidine products, the innovative solution for teat dipping is able to produce fly repellent and sun screen activity both suitable in summer. We see a high potential application of this solution in countries with specific environmental friendly strategy in the use of chemicals on farm. More studies on the actual mastitis prevention are currently carried out to harmonise the formulation before the actual availability on the market.

## BIOSECURITY AND ANIMAL HEALTH IN ORGANIC LIVESTOCK FARMING

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### SUMMARY

In order to evaluate the observance and effectiveness of biosecurity measures for organic livestock farms, a specially designed assessment file was sent to 42 county veterinary directorates to be filled in, following a thorough investigation. The evaluation of bovine and ovine organic farms with respect to the biosecurity measures applied, revealed that most of these organic farms have an adequate location, ensuring a good antiepidemical protection. The information thus obtained indicates that, in the light of current standards, the incidence of diseases in organic livestock farms is generally within acceptable limits, better than in conventional farms.

### INTRODUCTION

Romania has a remarkable agricultural potential: the agrarian surface is 14,8 million hectares, out of which 9,4 million hectares are arable (63%) and 4,9 million hectares are natural grazing fields (33%). Organic farming was initiated only 10 years ago /7; 9/, and its development began after 2000, together with the first legislative requirements inspired by the European legislation, according to international standards.

Organic farming aims at creating a sustainable agroecological system based on local resources and this is why it is developing nowadays at a fast pace. At present, there are 3033 registered producers in Romania: 121 producers have only vegetal products, 186 only keep one livestock species (182 only keep bees, two keep sheep and two keep laying hens), while the rest have a mixed agricultural and animal production.

Organic farming appeared as a consequence of people's distrust regarding food safety in conventional agriculture and also following the intensification of environmental and food pollution. Because consumers trust organic food production, biosecurity in organic livestock farms is a crucial matter. *Hovi* /2/ defines *biosecurity* or *animal health security* as the sum of the management measures to reduce the risk of introducing a new disease agent to the farm or of allowing existing disease agents to cause financial or health and welfare damage in the herd/flock. Our paper presents a first evaluation of biosecurity and risks from Romanian organic farms and it recommends several solutions for a better antiepzootical protection of organic farms, which may warrant the salubrity of organic products.

### MATERIALS AND METHODS

Data regarding livestock organic farming in Romania were obtained from the database of the Ministry of Agriculture regarding the producers, processors and tradesmen of organic products.

Out of the 3033 producers, 27 were selected. Criteria for the evaluation of biosecurity on these farms were included in an evaluation form. Questions were related to the identification and the type of the farm, the definition of the farm from an epizootical point of view, critical points of epizootical risk in that region, definition of risk from an environmental point of view, the system of antiepizootical protection, the qualification of health status on the farm, as well as other criteria. The evaluation form was sent to the county veterinary state authority to be filled in by field veterinarians, following an inspection of the farms.

Water samples were collected from nine farms and they were subjected to chemical and bacteriological laboratory tests. These samples, along with samples collected from other farms were also tested with the Microbiological Field Test /1/, for a comparison and evaluation of the utility of this test.

As requested by organic farmers confronted with the risk of mosquito attacks during rainy seasons, two biological products were also tested: *Vectolex* (spores of *B. sphaericus*) and *VectoBac* (spores and crystals of delta-endotoxin of *B. thuringiensis*). Laboratory tests were performed on mosquito larvae (*Culex pipiens*) that were introduced in various dilutions of the tested solutions. The active concentration and persistence time of the larvicidal effect were determined. Results were then checked under field conditions by applying biological products on the surface of swamps where mosquitoes are naturally reproducing.

Another tested product was *Oxygenon*, which contains hydrogen peroxide and peracetic acid. Tests were made on two strains of Gram negative bacteria (*E. coli* and *Ps. aeruginosa*), two strains of Gram positive bacteria (*St. aureus* and *B. cereus*) and two types of fungi (*Aspergillus* and *Saccharomyces*). Contact times were 15, 30, 60 and 120 minutes. Tests were performed in test tubes and on smooth and rough surfaces that were first sterilized and then contaminated with the microorganisms. The final evaluation of the product was made in a calf stall, which was disinfected and controlled by official sanitation tests, including the Coliform Bacteria Test and the Microbiological Field Test (MFT).

## RESULTS AND DISCUSSIONS

The study revealed several important aspects of Romanian organic livestock farming. The zotechnical profile of organic farms varies, but the great majority is either dairy farms or mixed farms with cows, sheep, goats and other species. The great majority of farms are small, with 5–20 cows and/or 300–500 sheep, with a small number of animals from other species. These farms produce fodder on their own land, which represents an important aspect of the antiepizootical protection.

Most farms are properly situated, 89% of them at a distance of at least 300 meters from other farms and 94,5% at a distance of at least 50 meters from village households. Farms are also situated at more than 22 meters from highly circulated roads. Farms are situated on plots of land with smooth slopes, protected from dominant winds and flooding.

Most farms are at a sufficient distance from the pollution sources, except for two farms, one of which is approximately 200m away from an aluminium plant, and another at a similar distance from a chemical storehouse.

Most organic farms (74%) have a protection fence with one or two entrances (11%); only one goat farm is not fenced (3.7%) and three farms have an interrupted fence.

An important deficiency is the fact that only 44.4% of the farms have their own pasture, while animals from the rest of the farms graze in the mountains (40.8%) or on the village pasture (14.8%), where other animals from conventional systems graze as well.

All farms have wells for the water of animals. The chemical and bacteriological water exam indicated that seven out of the nine examined wells provide water that is in perfect concordance with potability norms. The other two farms had a big number of coliform bacteria. The Microbiological Field Test provided results that were comparable to those obtained from laboratory exams, both for potable and non-potable water. As MFT is very easy to use, has a very low cost and can be used on farms, we recommend that this method be used on all farms and the laboratory microbiological exam be used only in particular situations.

As water can represent a source of diseases, one must take into account that, during summer, animals drink from various water-sources on pastures or nearby pastures.

The evaluation of biosecurity and risks on farms faces certain difficulties, as organic farm assurance legislation in the European Union (EU Regulation 2092/91 and 1804/99) does not say much about animal health security. National regulations are not sufficiently accurate regarding the methods of disinfections and insect and rodent control that should be used on farms. On the other hand, some of the farmers and veterinarians disregard the importance of investments that can insure biosecurity on farms.

There was a great variability in farm decontamination from one farm to another. Only 61% of the farms had a road disinfectant at their entrance, and even this is being used only during high epizootic risk periods, following the indications of the veterinarian. We found boots disinfectants in only 33% of the farms, and they were not continually used. A general annual disinfection is performed in 66.6% of the organic farms, and a general annual insect control is scheduled in half of the farms. No general disinfections of surfaces are made in the other farms, except for the whitewashing of walls.

Regarding the substances used for disinfections, it was noticed that farmers do not differentiate between certain disinfectants from the point of view of environmental pollution. The legislation is not clear regarding the categories of substances that are permitted for the decontamination of stables and of rooms where the first processing of animal products takes place. Based on the great efficiency of *Oxygenon*, and on the fact that hydrogen peroxide and peracetic acid are non-polluting agents, we consider that this category of products should be accepted for disinfections in organic farms. Microbiological laboratory tests, both those made in test tubes and those made on smooth and rough surfaces, have indicated that *Oxygenon* has antimicrobial properties against *St. aureus*, *B. cereus*, *Ps. aeruginosa* and *E. coli* at concentrations of 0.2%, 0.5 and 1%. The product was effective against fungi only at concentrations of 0.5% and 1%, in 15–30 minutes, at room temperature. The disinfectant effect on rough and absorbent surfaces was obtained with a solution of 1%. In the calf stable, *Oxygenon* 1%, 0.8 l/m<sup>2</sup>, for 30 minutes, provided a very good decontamination on all surfaces, including rough surfaces, at rather low temperatures (12–13°C). Tests for disinfection efficacy (Coliform Bacteria Test and Microbiological Field Test) provided similar results.

Biosecurity measures should not target only the decontamination of the environment and the supervision of disinfections' efficacy, but should also address the necessary technological refining of animal rearing systems and protection methods. An emphasis should be put on creating breeds that are highly resistant to usual pathogens. Some specialists have even signalled a potential conflict between short-term biosecurity and treatment measures, which are based on decontamination, together with isolation and treatment of sick animals and the long-term goal of positive animal health, which is aimed at obtaining robust, resistant, healthy animals. Another

important aspect is that biosecurity measures should not increase costs and hinder the development of positive animal health in a natural environment which addresses physiological needs.

The results of laboratory and field tests regarding the efficiency of *VectoBac* and *Vectolex*, indicated a good efficiency and a lack of environmental pollution. *Vectobac* produced the death of mosquito larvae at a dose of at least 127.9 ITU per ml, while *Vectolex* had this effect at a dose of 65 ITU per ml. The persistence of the larvicide effect, at a dose of 12,790 ITU per ml of water, was of about 3 weeks after the application. Siegel et al. /11/ have isolated *B. thuringiensis* from the product *Vectobac* and *B. sphaericus* from the product *Vectolex*, at an interval of nine months following their application in ponds. Moser et al /10/ tested *Vectolex* CG 7.5% under various field conditions and reported a mortality of mosquito larvae of 91%, when a dose of 2 g / m<sup>2</sup> was used and a persistence of 7 days of *B. thuringiensis* in water. The advantages of a bacteriological control of mosquitoes are that bacterial products do not affect people, animals and useful insects and they do not require special application methods and special work protection measures.

Biosecurity on farms is also very much influenced by the disposal of zootechnical residues (“animal by-products”). In all the organic farms that were evaluated, animals’ dejections are deposited on fermentation platforms and are used annually for the fertilization of their own agricultural land. Animal carcasses and other organic residues are thrown in constructed disposal pit (5.8% of farms), are buried in certain places outside the farm (41% of farms) or are collected by specialized companies and neutralized by industrial processing or burning. The regulations regarding the collection and neutralization of cadavers and other organic residues are still frequently disobeyed.

All the evaluated farms were considered free of the contagious diseases characteristic for that species. No zoonoses were noticed on the farms, except for ringworm in bovines. This appears occasionally because vaccination is not allowed on these farms. All farms benefit of qualified veterinary assistance. The veterinarian can be a permanent employee or a consultant. The regional official veterinarians also supervise these farms.

The evaluation forms indicate that morbidity and mortality does not differ very much from those registered on conventional farms. It is difficult to appreciate if the absence of major epizootical diseases is due to housing conditions, to the improved welfare and resistance of animals or to the fact that biosecurity measures are strictly applied in conventional farms, which surround all organic farms. Even if farm biosecurity is strictly correlated to regional and national biosecurity and depends on it, organic farms are exposed to other risks, derived from the breeding system. The main risk is represented by the fact that animals benefit of more freedom of movement and come into contact with various contamination sources.

Other researches show that internal parasitological diseases are more varied and intense in animals from organic farms, when compared to animals from conventional farms /4/.

On the other hand, organic farms are protected from contamination because they are situated at big distances from industrial plants and from conventional farms, because they are not allowed to buy animals or fodder from conventional farms and from livestock markets. However, the efficiency of antiepidemiological protection on farms depends a lot on health security “culture” and attitudes of farmers and on the recommendations of veterinarians.

The current regulations and standards for the control of diseases on organic farms recommend alternative medicine, based on herbal preparations, homeopathic medicine and acupuncture, which warrant the production of food with no medical residues /12/. It is still too early to jump to conclusions regarding the efficiency of such methods, but there are premises that unconventional medicine permits the same production as the conventional medicine /8/. Allopathic medicine is

admitted only in situations when alternative medicine are not efficient or when they require prolonged withdrawal periods for foodstuffs from treated animals.

Although specialists recommend various alternative methods with proven efficiency, these are little known in Romanian organic farms, because alternative veterinary medicine (phytotherapy, homeopathy, acupuncture) is not taught in Romanian veterinary schools. The use of various substances such as propolis or charcoal in a wrong way could cause adverse effects on animal welfare, consumer confidence or consumer protection /3; 5/. Farmers often distrust alternative therapy, because it requires multiple applications and a longer healing time, compared to conventional therapy. This is why farmers tend to use conventional methods, especially for parasite control. When these methods are not applied, there is an increase in the incidence of parasitological diseases, which was noticed in other countries, as well /6/.

### CONCLUSIONS

- 1) In spite of the marked development of organic farming in Romania over the last years, there is insufficient collaboration between competent authorities. On the one hand, inspection and certification bodies, which are legally accredited in Romania, do not provide enough support for the consolidation of this field. On the other hand, veterinarians are not prepared, professionally and organizationally, to provide services at international standards applied to organic farming.
- 2) Most organic farms do benefit of a proper location, which can ensure a basic antiepizoological protection, if hygiene rules are observed. Some farms do not comply with the mandatory fencing of animal stables, while others do not monitor properly the quality of their water sources, which can lead to insanitary food products.
- 3) The Microbiological Field Test (MFT) is recommended for the periodical supervision of local water sources and for the control of the efficiency of disinfection of surfaces and water. MFT can determine faecal pollution, is simple to use and has a low cost.
- 4) In view of mosquito control on organic farms we recommend bacteriological methods based on products that contain spores and toxins of *B. thuringiensis* or *B. sphericus*. These products do not represent health hazards for humans, animals and useful insects, such as bees.
- 5) We recommend the use of peracetic acid and hydrogen peroxide for disinfections in organic farms, because they have a very good disinfective potential, without polluting the environment.

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## MICROBIOLOGICAL AEROSOLS IN POULTRY HOUSES AND ITS AMBIENCE

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### ABSTRACT

The concentration of airborne bacteria, airborne *Escherichia coli* and airborne *enterococcus* in indoor air and downwind air at the distance of 10m, 50m, 100m, 200m, 400m and 600m of the farm were detected in order to find the influence to its ambience, using the Andersen-6 stages sampler and RCS sampler in five large chicken farms. The results showed that the culturable airborne bacteria concentration in indoor air of chicken house is  $3.80 \times 10^5 \square 2.57 \times 10^6$  CFU/m<sup>3</sup> air, which is higher than that in downwind air at the distance of 10m, 50m, 100m, 200m, 400m and 600m of the farm. The concentration of airborne *Escherichia coli* is  $0 \square 2.36 \times 10^2$  CFU/m<sup>3</sup> air in indoor air of the farm. In the same way, we have detected the concentration of culturable airborne *enterococcus* in indoor air is far higher than that in the downwind air outside of the farm. We have not detected airborne *Escherichia coli* and airborne *Enterococcus* in the distance of 400m even faraway outside of the downwind air. The airborne microbe concentration of all groups between the indoor air and in the air of the neighbourhood 10m, 50m, 100m, 200m, 400m and 600m from the house exhibited statistical significance or highly statistical significance ( $P < 0.05$  or  $P < 0.01$ ). No statistical significance between 10m, 50m, 100m 200m, 400m or 600m was observed. The results indicated that the microbiological aerosols in the chicken houses were relatively higher and were transmitted through the atmosphere to stable surroundings and over quite considerable distance (>100 m), especially the downwind air of the farm.

**Keywords:** microbiological aerosols, poultry houses, airborne *Escherichia coli*, airborne *Enterococcus*, spread of aerosol

### 1. INTRODUCTION

With the rapid development of stockbreeding, it has provided more livestock product for human being, and made huge contribution to resolving the problem of food and nourishment of population. And the modern agricultural methods have changed the way animals are raised (Donham, K. J., et al., 1977, 1982; Olson, D. K., et al., 1996; Steven M. Wolinsky, 2006). To increase production with minimum labor, chickens have been fed in confinement buildings, which are mainly enclosed structures densely stocked with chickens. A mechanical ventilation system and a system for handling animal wastes are usually set up to maintain the health status of chickens indoors.

However, the intensive livestock farming pollutes the environment of livestock farming itself, and affects the level of livestock farming. In the meantime, it pollutes the environment around,

and affects the living environment and living quality, restricts the positive and continuance development of stockbreeding. Microorganisms and their components or products, resulting from chickens dander, fecal matter, and feed materials, are easily accumulated and aerosolized in such densely populated and enclosed buildings (C. W. CHANG, et al., 2001). Due to exposure, chicken workers may experience upper respiratory irritation, chronic bronchitis, organic dust toxic syndrome, or other respiratory symptoms (Hagmar-L, et al. 1990; Wiegand-B, et al. 1993; Zucker-BA, Muller-W, 2000; Dennis Normile, 2004; Steven M. Wolinsky, 2006).

The kind and concentration of the microbiological aerosols is the indicative of the sanitations in animal house (Dutkiewicz-J, et al., 1994; Zucker-BA, S Trojan, et al., 2000; Zucker-BA, Muller-W, 2000; Kaliste-E, et al., 2002). The aim of this study was to detect the airborne bacteria, including the concentration of airborne aerobic bacteria and airborne *Escherichia coli*. and airborne *enterococcus* with Andersen-6 stages sampler and RCS in five chicken farms and their surroundings. We have detected the concentration of airborne bacteria, airborne *Escherichia coli*. and airborne *enterococcus* in the indoor air and the downwind air at the distance of 10m, 50m, 100m, 200m, 400m and 600m of the farm in order to find the influence to surroundings of the indoor airborne microorganisms.

## 2. MATERIALS AND METHODS

### 2.1 Animal houses studied

Air samples were collected during normal work periods in all poultry houses. Animal disturbance during sampling was strictly avoided. Five poultry houses were studied in this experiment. A description of these animal houses is given in table 1.

**Table 1.** Description of five poultry houses studied

	N	Layout	Inside			Outside		
			T(°C)	RH(%)	WS(m/s)	T(°C)	RH(%)	WS(m/s)
1	6000	Floor unit	26	40	0	21	50	1.0–3.0
2	2200	Cage unit	26	34	0	29	50	1.0–3.1
3	3000	Cage unit	31	44	0	35	36	1.5–3.0
4	3500	Cage unit	31	60	0	32	75	0–1.5
5	4500	Cage unit	30	70	0	31	65	0–2.0

**Note:** N=Number of poultry; T=Temperature; RH=Relative Humidity; WS=Wind Speed;

### 2.2 Airborne aerobic bacteria, *Escherichia coli* and *Enterococcus*

Six-stage Andersen samplers (Andersen, 1958) and RCS (Reuter Centrifugal Sampler, Biotest, Frankfurt) were used to collect airborne *E. coli* in animal houses and its surroundings (upwind 10m, 50m and downwind 10m, 50m, 100m, 200m, 400m). The samplers were located near the middle of the stable about 1.0m above the ground. The rate of airflow of Anderson sampler and RCS is 28.3L min<sup>-1</sup> and 40L min<sup>-1</sup> respectively. The samplers were equipped with MacConkey Agar No.3 (OXOID. LTD., BasingStoke, Hampshire, England) and 5% sheep blood agar and operated for 1 to 10 min according to the sanitation condition. The exposed agar plates were incubated at 37° C for 48 h. Bacteria were identified by Gram staining and then by using the API

system (Bio Merieux, Marcy-I'Etoile, France). Then the number of grown colonies were counted and the positive hole correction (Andersen, 1958) was applied.

Furthermore the concentration of airborne aerobic bacteria was determined outside of the poultry houses as described above. The samples were taken windward at a distance of 10m, 50m and leeward at a distance of 10m, 50m, 100m, 200m, 400m from the animal houses.

### 3. RESULTS

Table 2 shows that the total number of aerobic bacteria in the poultry houses was in the range of  $3.80 \times 10^5$ – $2.57 \times 10^6$  CFU/m<sup>3</sup> air; *E. coli* was in the range of 0–236 CFU/m<sup>3</sup> air; *Enterococcus* was 0–1131 CFU/m<sup>3</sup> air.

The total number of aerobic bacteria of upwind 10m and 50m from the poultry houses floated in the range of 480 to 7080 CFU/m<sup>3</sup> air; *E. coli* floated in range of 0 to 27 CFU/m<sup>3</sup> air; *Enterococcus* was 0–80 CFU/m<sup>3</sup> air.

The total number of aerobic bacteria of downwind 10m to 400m from the poultry houses was in the range of 684 to  $1.17 \times 10^6$  CFU/m<sup>3</sup> air; *E. coli* was 0–80 CFU/m<sup>3</sup> air; *Enterococcus* was 0–240 CFU/m<sup>3</sup> air.

The concentration and difference of airborne bacteria between the hen house and different distance in its neighborhood of 10, 50 and 100m were significant or highly significant ( $p < 0.05$  or  $p < 0.01$ ), while those in the neighborhood between 10, 50 and 100m showed no statistical significance ( $p > 0.05$ ).

**Table 2.** Concentrations of airborne aerobic bacteria, *Escherichia coli* and *Enterococcus* in indoor air and outdoor air of the 5 poultry houses (CFU/m<sup>3</sup> air). (n=5)

Poultry house	Airborne aerobic bacteria			Airborne <i>Escherichia coli</i>			Airborne <i>Enterococcus</i>			
	Max.	Min.	Median	Max.	Min.	Median	Max.	Min.	Median	
1	Upwind 50m	2534	510	1786	7	0	1	0	0	0
	Upwind 10m	3640	480	2184	11	0	2	0	0	0
	Indoor	216466	37951	89952	134	0	37	1131	0	230
	Downwind 10m	21760	2800	13952	49	0	12	71	0	14
	Downwind 50m	7560	1867	4297	24	0	6	0	0	0
	Downwind 100m	4400	1216	2263	11	0	3	0	0	0
	Downwind 200m	4302	894	1865	3	0	1	0	0	0
	Downwind 400m	3876	684	1708	0	0	0	0	0	0
2	Upwind 50m	1236	482	843	0	0	0	0	0	0
	Upwind 10m	1344	520	1030	0	0	0	0	0	0
	Indoor	82580	48975	80018	59	0	13	142	0	26
	Downwind 10m	29920	10320	17733	35	0	8	20	0	4
	Downwind 50m	6560	4880	5587	12	0	2	10	0	3
	Downwind 100m	6760	3160	4730	0	0	0	10	0	3
	Downwind 200m	2453	1220	1994	0	0	0	10	0	2
	Downwind 400m	1480	720	1118	0	0	0	0	0	0

**Table 2.** Continuation

Poultry house		Airborne aerobic bacteria			Airborne <i>Escherichia coli</i>			Airborne <i>Enterococcus</i>		
		Max.	Min.	Median	Max.	Min.	Median	Max.	Min.	Median
3	Upwind 50m	1896	583	1023	0	0	0	0	0	0
	Upwind 10m	2451	826	1894	0	0	0	0	0	0
	Indoor	182862	110530	143173	71	0	14	671	0	143
	Downwind 10m	28646	7654	22760	12	0	3	35	0	7
	Downwind 50m	7560	3864	5684	0	0	0	0	0	0
	Downwind 100m	6760	3160	3750	0	0	0	80	0	16
	Downwind 200m	2556	1234	2994	0	0	0	0	0	0
	Downwind 400m	2480	976	2019	0	0	0	0	0	0
4	Upwind 50m	5760	890	3248	10	0	2	0	0	0
	Upwind 10m	7080	3100	5027	27	0	7	80	0	16
	Indoor	257279	84488	148919	236	0	63	424	0	87
	Downwind 10m	29040	23440	26688	80	0	24	80	0	16
	Downwind 50m	8400	3920	5888	40	0	16	10	0	3
	Downwind 100m	11560	6880	9632	10	0	2	0	0	0
	Downwind 200m	10246	5810	8764	10	0	2	0	0	0
	Downwind 400m	4123	2947	3278	0	0	0	0	0	0
5	Upwind 50m	2480	1520	2080	0	0	0	0	0	0
	Upwind 10m	2982	1630	2458	0	0	0	0	0	0
	Indoor	192721	158021	170389	35	0	9	495	0	108
	Downwind 10m	24480	5680	14293	18	0	5	240	0	48
	Downwind 50m	7840	2840	5800	0	0	0	0	0	0
	Downwind 100m	116960	91120	100747	0	0	0	144	0	31
	Downwind 200m	43520	25387	32338	0	0	0	10	0	2
	Downwind 400m	5120	3547	4276	0	0	0	0	0	0

#### 4. DISCUSSION

It is well known that there are many types and strains of *E. coli*, a few of which are potentially pathogenic. Various strains may cause illness by a variety of infective and toxin-producing mechanisms. In poultry, *E. coli* can cause many diseases such as septicemia, swollen head syndrome, omphalitis, cellulitis, yolk-sack infection and respiratory tract infections (Sojka and Carnaghan, 1961; Morley and Thomson, 1984; Randall et al., 1984; Dho-Moulin and Fairbrother, 1999). The resulting morbidity and mortality have led to serious economic losses to the poultry industry (Gross, 1994). And it can cause many diseases to human beings, such as hemolyticuremic syndrome (HUS), haemorrhagic colitis (HC), neonatal meningitis and bloody diarrhea (Marjut Eklund, et al., 2001; Norval J.C. et al., 2005; S.K. Manna, et al., 2006; Marilda C. Vidotto, et al., 2007). In addition, *Enterococcus* is a kind of common bacteria in the air of animal house environment too (Cormier Y, et al., 1990; Crook B, et al., 1991; Predicala BZ, et al., 2002). And *Enterococcus*, particularly some of the species including *E. faecalis* and *E. faecium*, is indigenous flora in the human bowel and has emerged as one of the leading causes of nosocomial bacteremias, urinary tract infections, central nervous system destroy, and wound infections (Uttley et al., 1998; Satoshi Takahashi, et al., 1999; Gambarotto et al., 2000; Soltani et al., 2000; NNIS 2001; É.J. Kaszanyitzky, et al., 2007).

In this study, the concentration of aerobes, *E. coli* and *Enterococcus* in indoor air of poultry houses was much higher than its ambient. In the open-air there was only a small amount of *E. coli* and *Enterococcus* normally (Yu, xihua and Fx. Che, 1997). Therefore, microorganism in the air of the neighborhood of the hen house came from the indoor air of the poultry house. It can be concluded that the concentration of the bacteria aerosols in the hen house was slightly higher than normal. So, such a high concentration of bacteria particles in the indoor air means that the animals must have had some diseases, were in recessive infection or were germ carriers. Animals breeding in high density will lead to building a quicker channel exchanging of pathogen bacteria within animals, and the pathogenicity of the bacteria will be raised (Melhorn and Chai, 2000). This suggested that the microbiological aerosols in the chicken house could be transmitted through the atmosphere to stable surroundings over quite considerable distance and could cause environment pollution as well as spread of epidemics. And the presence of high concentrations of airborne culturable bacteria and potentially allergic might pose health risks for workers (C. W. CHANG, et al., 2001). So, poultry house should be set up outside 400 meters away from resident at least. These results can provide important reference for the officers and farm keeper.

Although there is no statistical data which can prove the fact that there is some inherent correlation between the concentration and the incidence of the disease, from many studies we infer that the high concentration of the airborne microorganism can burden the immune system of the animals, make them grow slowly and reduce their economic value. Chai (1998) concluded that the concentration in the cowshed with straw should not exceed  $10^4$  CFU/m<sup>3</sup> and in the hen house should not exceed  $10^4$  CFU/m<sup>3</sup>, though there is no unitive criterion about the concentration of the airborne particles in the hen environments. In the present study, the concentration of the aerobes in indoor air, upwind air and downwind air of poultry houses were  $3.80 \times 10^5$ – $2.57 \times 10^6$  CFU/m<sup>3</sup>air, 480 to 7080 CFU/m<sup>3</sup>air and 684 to  $1.17 \times 10^6$  CFU/m<sup>3</sup>air respectively, which are very higher than that in the normal plain (Yu and Che, 1997). So the hygienic condition of the poultry house should be improved.

### ACKNOWLEDGEMENTS

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## AMMONIA TREATMENT OF HATCHERY WASTE FOR ELIMINATION OF AVIAN INFLUENZA VIRUS

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### SUMMARY

The inactivating capacity of ammonia to the avian influenza virus (H5N3) was investigated, using influenza virus spiked hatchery waste and ammonia to final concentrations of 0.25, 0.5 and 0.75% (w/w), at a temperature of 15°C. As control hatchery waste with only deionised water was used. To evaluate the possible use of bacteriophages as indicator organisms, the hatchery waste was challenged with Enterobacteria phages MS2 and  $\phi$ X174 to monitor the inactivation. The results indicate that avian influenza virus H5N3 is readily inactivated at all ammonia concentrations tested. Bacteriophage  $\phi$ X174 did not show any significant decay at any of the ammonia concentrations, whereas bacteriophage MS2 was inactivated at a slower rate than influenza virus. Ammonia is a good alternative for inactivation of avian influenza virus in hatchery waste, and bacteriophage MS2 can be used as a conservative indicator.

**Keywords:** avian influenza virus; ammonia sanitation; inactivation; hatchery waste

### INTRODUCTION

Hatchery waste (HW) is produced by the hatchery industry and may contain pathogenic microorganisms, e.g. viruses. According to the regulation (EC) 1774/2002, which lays down health rules for animal by-products not intended for human consumption, the HW needs sanitation treatment. The most common method to do this in Sweden today is by liming. However, liming is technically complicated and can lead to unsuitable working conditions, e.g. due to the high pH (>12), lime dust and formation of sediments. In the case of an epizootic disease outbreak, a suitable environmentally safe sanitation method would be desirable, instead of e.g. sodium hydroxide or formaldehyde treatments now recommended for use in Sweden (1). Ammonia sanitation of biowaste can be performed by using ammonia (aq) or urea (s), which has been studied regarding faeces (2). Free uncharged ammonia ( $\text{NH}_3$  (aq)) is the active substance and is present at pH values >8 with a pKa of 9.3. The equilibrium of  $\text{NH}_3$  and its ionised form  $\text{NH}_4^+$  depends on both pH and temperature and is moved towards  $\text{NH}_3$  when either of them is increased. Studies have indicated that  $\text{NH}_3$  acts by cleavage of viral RNA; this has been shown regarding poliovirus, a picornavirus which possess single stranded (ss) RNA, at a temperature range of 10 to 40°C (3). As influenza virus also possesses ssRNA it could be expected that it would be sensitive to ammonia. The ammonia treatment contributes further to the agricultural fertiliser value of the biowaste, as ammonia persists during the treatment, and therefore may be used as a plant nutrient.



## OBJECTIVE

The objective of this study was to investigate, in laboratory scale, the potential of using ammonia as a chemical treatment method for disinfection of HW. A second objective was to evaluate usage of bacteriophages as monitoring models for virus inactivation.

## MATERIAL AND METHODS

The material used for the disinfection studies was untreated hatchery waste (HW) obtained from Lantmännen SweHatch AB, Kristianstad, Sweden. The HW consisted of egg shells, egg yolk and chicken embryos, and was stored in aliquots at 4–6 °C for 2 weeks before experimental start.

Disinfection of the HW was monitored by analysis of viable added microorganisms. The viruses used were avian influenza A virus (AIV), strain H5N3, isolated at Department of Virology, SVA. The bacteriophages used were Enterobacteria phage MS2 (ATCC 15597-B1) and Enterobacteria phage  $\phi$ X174. Cell culture medium for AIV was Eagle's minimal essential media (4) (EMEM) containing trypsin (Worthington Biochemical Corporation, Lakewood, NJ, USA) at a concentration of 2.5  $\mu$ g/ml, and cell line was Madin Darby Canine Kidney (MDCK) cells (ATCC CCL-34).

HW in 1-g portions was spiked 10-fold with the respective microorganism to an initial concentration of 5–7  $\log_{10}$  per gram. Ammonia (Rectapur, PROLABO, Stockholm, Sweden) was added to final concentrations of 0.25, 0.5 or 0.75%(w/w) to the spiked HW, by thoroughly vortexing for complete homogenisation. As controls, spiked HW with only deionised water was analysed, to determine the inactivating effect of the HW and temperature alone. For comparison, pure virus suspension was kept at the actual temperature and time periods. All mixtures were treated at 15 $\pm$ 0.5 °C, the temperature and pH was monitored during the treatment. Sampling was performed 4–5 times for each ammonia inactivation trial. At each sampling two 1-g samples of HW were taken, diluted 10-fold in cell culture media, and extracted by vigorous shaking. After centrifugation at 3000 g for 10 min, the supernatant was gel filtrated through Sephadex G-25 columns (GE Healthcare, Uppsala, Sweden) in cell culture media, to remove ammonia and other cytotoxic low molecular weight substances.

To assess the detection limit before virus analysis, a viral interference assay was performed: HW with the highest ammonia concentration was extracted as above, and subjected to gel filtration. AIV was titrated in the resulting effluent, and the virus titre was compared to virus titres obtained using cell culture medium only as titration media.

Virus was analysed by an end-point titration method through cell culture cytopathic effect using eight 50- $\mu$ l replicates per dilution, and the virus titres were calculated according to the Spearman Kärber formula (5) and expressed as tissue culture infectious dose (TCID)<sub>50</sub> values per gram HW. The virus reduction factors where no virus was found were calculated according to (6). The double agar layer method (7) was used to determine the number of the bacteriophages. For MS2, the *Salmonella enterica* strain WG49 (ATCC 700730) was used as the host bacterium, and for  $\phi$ X174 the *Escherichia coli* strain C (ATCC 13706™) was used. The bacteriophage titres were expressed as plaque forming units (PFU) per gram HW.

## RESULTS

The pH of the HW with 0, 0.25, 0.5 and 0.75%(w/w) ammonia added was 7.5, 8.5, 9.0 and 9.0–9.5, respectively, and was not altered up to 18 hours of treatment. The results from the viral interference assay for AIV showed that the undiluted, gel filtrated samples could be used for virus analysis. The titres from the analysis of the microorganisms are shown in Table 1.

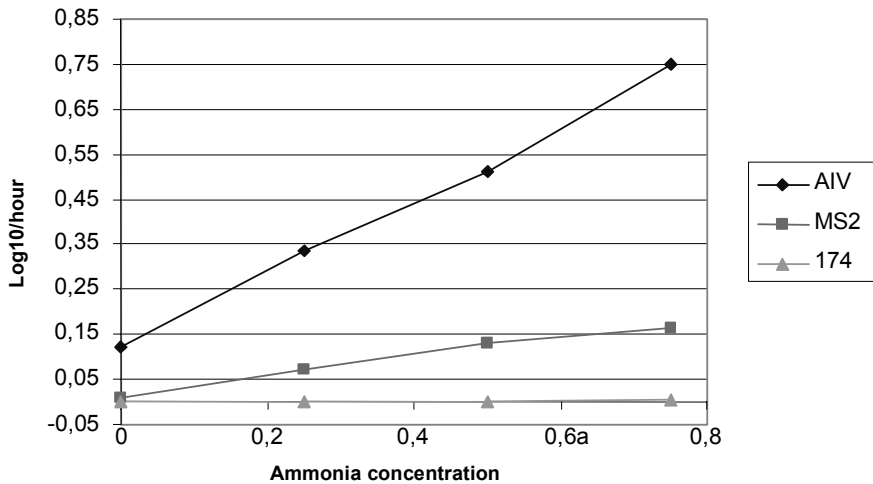
**Table 1.**

Microorganism/ NH <sub>3</sub> %	0	3	6	Time 8	Hours 12	18	29	72
AIV 0	5.4	5	4.7	4.5	3.8	a	a	a
AIV 0.25	5.4	3.6	3.4	3.0	<0.9	a	a	a
AIV 0.5	5.4	2.9	2.5	<0.9		a	a	a
AIV 0.75	5.4	2.9	<0.9			a	a	a
MS2 0	5.9	a	5.8	a	a	5.8	5.5	5.4
MS2 0.25	5.9	a	5.5	a	a	4.6	3.6	<0.9
MS2 0.5	5.9	a	4.8	a	a	2.9	2.0	<0.9
MS2 0.75	5.9	a	3.6	a	a	2.5	<0.9	<0.9
φX174 0	7.0	a	7.0	a	a	7.0	7.0	7.0
φX174 0.25	7.0	a	6.9	a	a	7.1	7.0	7.0
φX174 0.5	7.0	a	7.0	a	a	6.9	6.9	6.9
φX174 0.75	7.1	a	7.1	a	a	6.8	7.1	6.8

Mean titres in log<sub>10</sub> tissue culture infectious dose (TCID)<sub>50</sub> (AIV) or plaque forming units (PFU) (bacteriophages) per gram HW. Detection limit was 0.9 log<sub>10</sub>

<sup>a</sup> = not tested

As can be seen in Table 1, AIV was inactivated to below detection limit after 6–12 hours at 0,25–0,75% NH<sub>3</sub> concentrations, a virus reduction of >4.5 log<sub>10</sub>. Concerning MS2, complete reduction of >5.0 log<sub>10</sub> was evident after 29 hours at 0.75% NH<sub>3</sub> and after 72 hours for 0.25–0.5% NH<sub>3</sub>. φX174 was not significantly inactivated at any ammonia concentration. The controls kept in cell culture media were inactivated about 1 log<sub>10</sub> after 12 hours for AIV, and about 0.5 log<sub>10</sub> for MS2 after 72 hours. φX174 control in cell culture media was inactivated by about 1 log<sub>10</sub> after 72 hours.



**Figure 1.** Inactivation rates in log<sub>10</sub> per hour of avian influenza virus H5N3 (AIV), Enterobacteria phage MS2 (MS2) and Enterobacteria phage  $\phi$ X174 (174) at different ammonia concentrations at 15°C.

As can be seen in figure 1, the inactivation rates of AIV are higher than for MS2 and  $\phi$ X174 at all ammonia concentrations, indicating that the bacteriophages investigated are more stable.

## DISCUSSION

Studies regarding Enterobacteria phage f2, a phage of the same virus family and genus as Enterobacteria phage MS2, showed that f2 is about 4.5 times more resistant to ammonia inactivation as poliovirus, an enterovirus of the virus family Picornaviridae, both possessing ssRNA (8). In our study the ssRNA phage MS2 was 4–5 times more resistant to ammonia inactivation than AIV. Influenza virus is also more sensitive to environmental factors due to its lipid envelope, and is inactivated at pH >9.5, a lower pH than for enteroviruses such as poliovirus which is inactivated at pH  $\geq$ 11 (9).

The bacteriophage  $\phi$ X174 was not inactivated significantly at any of the ammonia concentrations used, during the whole time span of the trial, 72h.  $\phi$ X174 possess circular ssDNA that could make it more resistant to NH<sub>3</sub> inactivation, as it is otherwise quite similar to bacteriophage MS2, generally DNA is more stable compared to RNA.

AIV in the controls of HW with deionised water at pH 7.5 was reduced by 1.6 log<sub>10</sub> after 12 hours, due to intrinsic properties of the hatchery waste. Results reported in earlier studies on swine influenza A virus show that at 5°C and 20°C, the survival in cattle slurry is 9 and 2 weeks, respectively, a survival comparable to picornavirus at 20°C (10). Avian influenza virus (H7N2) in chicken manure was reported to be inactivated in less than one week at 15–20°C (11). Thus both temperature and properties of different vehicles might influence influenza virus survival.

It can be concluded that avian influenza virus (H5N3) is readily inactivated by ammonia treatment. After 12 hours in the lowest ammonia concentration used, more than the requested 3

$\log_{10}$  reduction was achieved. The MS2 phage showed a lower reduction rate than AIV; therefore it can be used as a conservative indicator for inactivation of AIV, as one  $\log_{10}$  reduction in MS2 corresponds to at least three  $\log_{10}$  reduction of AIV.

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## THE UTILIZATION OF WIND-TUNNEL TO ESTIMATE THE DUST CONTENT OF CHOSEN BEDDING MATERIALS

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### SUMMARY

The aim of carried searching was to define the dust content of bedding material, as well as new alternative litter supplements, tested in respect of sorption level of toxic gasses, that are commonly used in technology of animals' husbandry on deep litter. Tested was sawdust of conifers, pulverized haloisit (HAL), wheat strowe (WS), pellets feed for chicken (PF) and *Miscanthus sinensis* strowe (sMs). The dusting was performed by using of wind-tunnel, congenial in shape to the Witoszyński-nozzle, what allows obtaining an equal velocity distribution in inlet profile. Such shape of this part has a task to determine the flow velocity in the whole tunnel profile and to eliminate possible turbulences, arising by the inlet. The surface of material tested in the tunnel, as a source of dusting, amounted to 0.09 m<sup>2</sup>. In each of tested bedding materials, before performing the test for the level of dust emission, there was defined the water capacity. Tests were performed for 4 velocity ranges of air flow through the tunnel, 0,15, 0,2, 0,3 and 0,5–0,7 m/s. The level of dusting was estimated by using of aspirator AP-2000EX (PN-91-Z-04030/05), being in use to collect samples of air contaminated with industrial dusts from the breathing zone of the laborer on his post, as well as other searching in the flowing range of 0,6 ÷ 2,2 l/min. The collection of air sample from the tunnel was performed by the flowing of 2 l/min.

Each of tested materials has emitted dust by the air movement velocity of 0,3 m<sup>2</sup>, except of haloisit, that was dusting already by the air flow velocity of 0,15 m/s. Haloisit also characterized itself with the highest level of dusting.

**Keywords:** wind-tunnel, dust emission, strowe, haloisyt, pellets feed

### INTRODUCTION

Near by such important for animal health adverse admixtures in the air of farm accommodation as ammonia, hydrogen sulphide or number of microorganisms mentioned is also dust. Basically, in the qualification, there are distinguished molecules with diameter to 5 µm, defined as respirable dust, on the contrary to molecules with diameter over this value – as sedimentative dust [Dobrzyński 1999]. The high level of air dust in livestock rooms acts disadvantageous on the animal health [Dobrzański and Kołacz 1996]. Dust with small diameter penetrates through the mucosa and lungs to the blood flow and can cause arising of pneumoconiosis by the animals. Dust of bigger size settles on the mucosa of upper air passages and conjunctiva, effecting their irritation

and damage. The consequence might be inflammatory state and damaging of natural barrier which mucosa or skin for pathogenic microorganisms are.

Air dust in the rooms for poultry, especially broilers held in the litter system is very high. Wathes at all [1998] have stated medium air dust in broiler room with floor litter system with respiratory dust on the level of  $3,6 \text{ mg/m}^3$  and with inhalative dust in amount of  $0,45 \text{ mg/m}^3$  and in the summer time the air dust level was 40% higher than in the winter time.

Near by seasonal changes of air dust level in livestock rooms, Hinz and Linke [1998] have noted daily fluctuation. They have determined the level of air dust in the fattening building-during the night on the level to  $2 \text{ mg/m}^3$ , during the day on middle level from 1 to  $5,5 \text{ mg/m}^3$ , additionally they have observed sudden rise of air dust during meal of fatteners to the levels containing in the range from 15 even to  $25 \text{ mg/m}^3$ , what is strictly connected with the activity rise of animals. Usually searching concerning the level of air dust in rooms for poultry and swine, held in litter system, consisted in determination of air dust condition in these structures, depending on litter materials, which were in use and in using of different methods to limit the dusting intensity of litter. Few are reports about *in vitro* tests, with using of wind tunnels to determine the dusting level of different materials being in use as litter for animals or such materials that can be alternative for general used materials. Recognizing of dusting level of the litter material before its application seems to be justified because of the fact that the rise of farm buildings number increases global dust emission to the atmosphere. Report in form of stocktaking of contaminants emission to the atmosphere, worked out by the Institute of Environment Protection, National Centre for Emission Inventory Control, for 2001 [2003] shows estimative, that in Poland dust emission of agricultural descent to the atmosphere, makes 5% of global emission. From this 97,6% (23 524,4 tons) is dust derived from animal production, exactly from processes on the field of animal faeces economy. Robertson at all [2002], have stated during the searching of 4 farm buildings for broilers in Great Britain, among other things in regard of dust emission to the atmosphere, that emission per hour from 1st till 28th or 30th day from settling of the building had risen from the level of  $0\text{--}5 \text{ g/m}^3$  of dust to  $252\text{--}505 \text{ g/m}^3$  that confirms the high level of dust emission from big farm structures to the atmosphere.

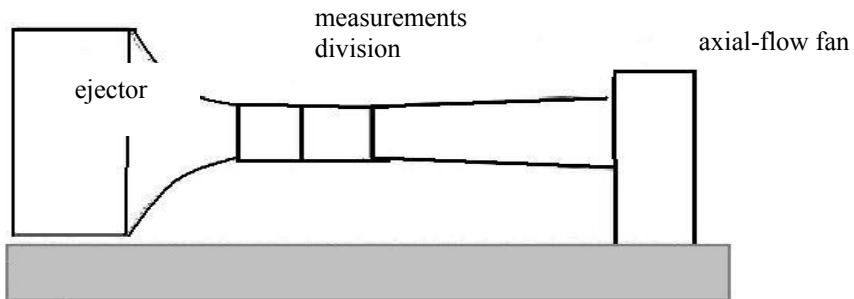
## MATERIAL AND METHODS

In the searching tested were, in regard of dusting, litter materials of general use by poultry farming and breeding (wheat straw – WS, straw *Miscanthus sinensis* -sMS), feed for poultry in granulated form (PF) as well as halloisite (HAL), used as bedding supplement to limit the emission of injurious gases to the air of livestock house.

The dusting intensity of studied materials was defined for given initial humidity of the material and by fixed air movement speed (0,15; 0,2 0,3 and 0,5 m/s). Every dusting has lasted 15 minutes. Additionally after the ending of the 1st stage there have been performed dustings of these materials in the period of 60 minutes for the air movement speed of 0,6 m/s. Dobrzański [1999] gives, that air dusting in the rooms for poultry shouldn't cross the value from 1 to  $3 \text{ mg/m}^3$ , depending on the keeping system. Wathes [1994] gives, that it can reach the limit to  $1,7 \text{ mg/m}^3$  of respiratory non-specific dust.

For the dusting test there was used a wind tunnel. The tunnel is a construction with total length of 5 m and square cross-section. The inlet part of the tunnel, made of sheet metal plate, has similar form to Witoszyński's nozzle, what allows obtaining an equal speed disposition in the inlet section. Such form of this part has a task to stabilize the flow speed in the whole tunnel section

and eliminate of possible turbulences arising by the inlet. Next element of the wind tunnel is the measurement section of dimensions 300x300 mm, made of transparent perspex (enabling observations of the effect course). The researched sample was placed in special formed cut-out in the tunnel base. It causes, that the sample surface is situated on the same level as the base of the installation. The cut-out allows placing the sample of dimensions 300x300x50 mm. Further the air is carried away through the 1,5 m long tunnel segment by using of axial-flow fan. In front of the cell with sample a measurement of the air stream speed was made. The measurement was made in assistance of hot-wire anemometer, which can be optionally relocate both in the vertical and horizontal surface. For assurance of fluently speed regulation of the air stream flow, the fan engine was connected to the frequency current converter. It allowed achieving an effective speed of the air flow in the tunnel, in the range from 0 to 3 m/s. The measurement place was also equipped with additional devices for measurements of partial vacuum in the measurements section, enabling its calibration.



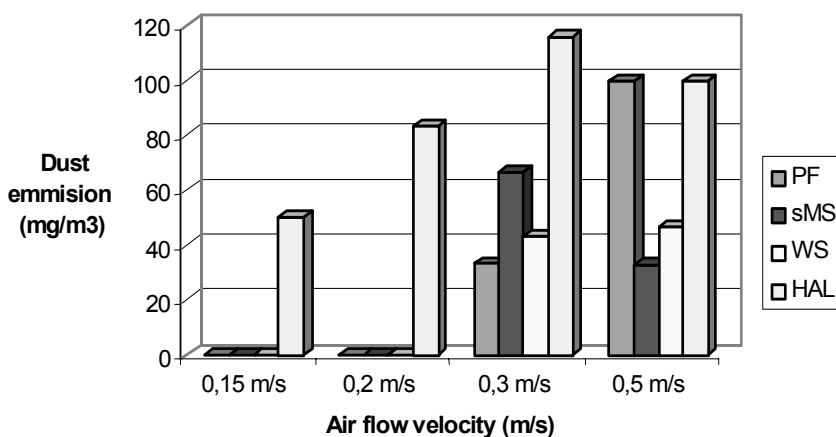
**Figure 1.** Ideological scheme of the measurements place – wind tunnel

The determination of the conditions in the wind tunnel – flow speed, was carried out in accordance with polish standard PN – ISO 5221 from December 1994 “Air distribution and separation. Measurement methods of the air stream flow in the conduit”. The dusting intensity was defined in assistance of Aspirator AP-2000EX, serving to drawing of air samples, impure by the industrial dust from the respiration zone of the laboratory assistant on his work-stand and other tests from the range of the flow from 0,6 to 2,21/min.

## RESULTS AND DISCUSSION

Researched materials were characterised by similar humidity from 12,9% in granulated feed (PF) to 9,9% in wheat straw (WS).

In the consequence of dusting test there was stated, that by the lowest speed of air movement – 0,15 m/s only mineral sorbent – halloysite (HA) has dusted. The dust emission from halloysite by this air movement speed amounted to 50 mg/m<sup>3</sup> (fig. 2). By the air movement speed of 0,2 m/s dust emission only for halloysite was still noted. The amount of dust emitted from halloysite has risen of over 30 mg/m<sup>3</sup>. On the ground of over presented results it was stated that humidity of this material should be raised to limit its emissive properties. Such a test wasn't undertaken because potential material use of this kind in animal production has in view to utilize their sorptive properties in regard of limitation of odour formative gases emission from the litter. Every quantity growth of water in researched materials would cause decrease of their sorptive potential.



**Figure 2.** Dusting level of researched materials by determinate speed of air movement in the tunnel.

Defining the emission level of total dust from researched materials by the highest air movement speed in livestock houses, recommended in the winter time (0,3 m/s), there was stated, that the biggest dust amount provided halloysite, in quantity of 116,16 mg/m, then straw *Miscanthus sinensis* – sMS, on the level of 50–60 mg/m<sup>3</sup>. In case of other materials emission was on the level of about 30 mg/m<sup>3</sup>. For the air movement speed of 0,5 m/s (the highest acceptable in the summer time in the livestock buildings) no substantial changes in the quantity of emitted dust for straw and halloysite was stated. In case of the test of 0,5 m/s for granulated feed threefold growth of dust amount in comparison to the test for air movement in the tunnel, on the level of 0,3 m/s, was noted.

An exception was also the Chinese *Miscanthus*. The quantity of dust supplied by this material has been reduced of 50% in relation to test performed by the speed of 0,3 m/s. That chance results very likely from great heterogeneity of the material that is why the fine elements were left on the bottom of the cell and big parts of stalks has covered them.

On the grounds of obtained results it can be stated, that the biggest dust emission during the research has characterised the halloysite, hence its usage as a supplement for litter to limit the emission of toxic gases to the air can enlarge the air dusting of livestock rooms. From among of tested materials, used commonly in the technology of poultry production, the greatest “deliverer” of dust to the room air, is proved to be the feed, especially by higher speeds of the air movement. Nakaue at all [1981], making studies on the quantity of elements identified in the dust from the air of layer hen room have stated, that the feed is one of main air dusting sources in buildings of cage-keeping system. Litter materials can be also serious dust emitter in the livestock rooms what confirm studies of Dobrzański at all [1988]. On the basis of obtained results the authors have acknowledged litter as main dust source in the rooms for poultry, held on bedding, and the number of microorganisms in the air of such room is conditional on the level of its dusting. Additionally it was stated, that the air dusting size in rooms of this kind depends on the air humidity, temperature, but above all from activity of birds which are held there.



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## SOILING AND CLEANING OF FLOORINGS IN ANIMAL HOUSES

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### SUMMARY

Although soiling and wearing of surfaces in animal buildings is a major problem in animal production, only a limited number of investigations of these phenomena have been published. However, the surfaces should be clean and at some sites hygienic e.g. in order to guarantee product quality. Furthermore, there are no common standard procedures used for monitoring hygienic quality of surfaces in animal houses. In the present literature survey, methods for measuring surface properties, wearing and cleanability of surfaces for use in animal houses are introduced and evaluated. In addition, different cleaning and soiling systems are reviewed.

**Keywords:** soil, detection methods, animal house, piggery, cowhouse

### SOILING OF FLOOR SURFACES IN ANIMAL HOUSES

Typical surface materials in animal houses are presented in Table 1. The surfaces in agricultural buildings are subjected to several contaminants, e.g. mixtures of manure, feed and washing waters (Table 2).

**Table 1.** Typical surface materials used in animal houses

<b>Room</b>	<b>Frequently used surface materials</b>
Walking and laying areas	Concrete, asphalt, rubber mat, stall mattress
Feeding troughs	Special concrete, epoxy, grinded mass
Milking station	Concrete (roughened coatings), ceramic tiles, acrylic concrete, epoxy
Milk room	Concrete, ceramic tiles, acrylic concrete, epoxy
Assistant rooms	Concrete (painted), plastic flooring, acrylic concrete, ceramic tiles

**Table 2.** Examples of typical contaminants and chemical substances in the floorings in animal houses, mentioned in the literature. The main sources of the harmful substances are manure and feed residues.

Harmful substance	Reference
Acetic acid	DeBelie et al. 1996, ACI 515.1R-79 1985
Butyric acid	Bertron et al. 2005, Nilsson 2005
Capronic acid	Mathiasson et al. 1991
Lactic acid	DeBelie et al. 1996 & 2000
Propionic acid	Bertron et al. 2005
Valerian acid	Mathiasson et al. 1991
Chlorides and sulphides	Calleja Carrete 2005
Aggressive ions $\text{NH}_4^+$ , $\text{Mg}^{2+}$ , $\text{Cl}^-$ and $\text{SO}_4^{2-}$	DeBelie et al. 1996

Manure acidifies over time. Regular and efficient removal of manure when fresh thus helps prevent concrete from becoming brittle (Mathiasson et al., 1991). Similarly to acetic acid, lactic acid also weakens concrete slowly (standard ACI 515.1R, 1985). In a study by De Belie (1997) the feeding method of pigs had the greatest impact on erosion of floorings, the most severe damage being at the liquid feeding site.

## MECHANICAL AND STRUCTURAL SOLUTIONS FOR PREVENTING SOILING AND REMOVAL OF SOIL

The floor construction can be solid or slatted. A solid floor is combined with an open or covered drain, whereas a slatted floor requires a collecting system for liquid manure under the floor (De Belie, 2000b). Slatted floors can normally be kept cleaner than the solid floors. A rubber slatted floor caused less soiling and injuries to cows compared to cows on a solid floor in a study by Hultgren and Bergsten (2001). If the inclination (slope) of the floor is 7%, the floor is still comfortable enough for the cows (Nørgaard et al. 2003), but helps the flooring to stay dry and reduces the need for cleaning (McClanahan 2005).

## CLEANING METHODS

The manure funnels are normally cleaned mechanically with a scraper, and the feeding troughs with a brush or a rake. Regular pressure cleaning with a pressure of 80–100 bar (Böhm 1998) is the most common cleaning method currently used for floors in animal houses (DeBelie et al. 2000a). Running water from a hosepipe is also a typical means of cleaning animal houses. According to Böhm (1998), the surface should ideally be soaked for 1–2 h before the actual cleaning but this is only rarely the case. In order to make cleaning easier, Larsson (2000) recommended a soaking time as long as 24 h prior to cleaning, but this is rather unrealistic. In the study by Larsson (2000) a washing robot consisting of pressure cleaning and an electronic control system was tested in the cleaning of pig pens. An advantage of using the robot was reduction in manual work, whereas water consumption increased.

Detergents are used only at special sites in cowhouses, such as for cleaning of milking robots and floorings of the milking area. Detergents are also not usually used in cleaning of floorings of piggeries.

### DETECTION METHODS FOR CLEANNES OF SURFACES

In experimental studies, visual and qualitative evaluation methods have mainly been used. An evaluation of different qualitative, semi-quantitative and quantitative methods is presented in Table 3.

**Table 3.** Detection methods of cleanability and evaluation of methods

Detection method	Reference	Evaluation of the method
Visual evaluation	Sundahl 1974, Hörndahl 1995, Puumala&Lehtiniemi 1993	Subjective, qualitative. Requires a large group of evaluators if properly used. Suitable for field and laboratory studies.
Colorimetry	Kymäläinen et al. 2007	Semi-quantitative. Detects visible soil on surfaces. Suitable for field and laboratory studies.
Other optical methods (visual and near-infrared optical range)	Zhang et al. 2006	Semi-quantitative. The spectral signals can be used for discrimination of dirty and clean conditions of the surfaces.
Chemical and biochemical tests	Larsson 2000	Detects (visible and) invisible organic soil on surfaces. Often sensitive, suitable for clean sites. Suitable for field and laboratory studies.
Microbiological methods	Larsson 2000, Pelletier et al. 2002	Detects (visible and) invisible soil on surfaces. Suitable for field and laboratory studies.
Radiochemistry	Kymäläinen et al. 2007, Määttä et al. 2007	Quantitative. Detects visible and invisible soil both on and absorbed in the surface. Only for laboratory use, requires special equipment.

### WEARING OF MATERIALS

Both chemical substances and mechanical impact on floorings cause corrosion and wearing that may promote injuries to the animals. In addition they may make cleaning difficult, thus promoting spreading of diseases (DeBelie 1997, DeBelie et al. 2000). In practice, animals and high pressure cleaning both cause mechanical wear in the flooring (Mathiasson et al. 1991, O'Donnell et al. 1993, ACI 515-1R-79 1993, DeBelie et al. 1996, DeBelie 1997, DeBelie et al. 2000a&b, Calleja Carrete 2005), which may increase both its roughness and the space between the slats. However, in studies by Barnes (1979) and De Belie (1997), high pressure cleaning did not intensify the erosion of concrete flooring. According to Barnes (1979), a pressure of 7 N/mm (7 MPa) may wear the surface of low-quality concrete. According to De Belie (1997), detergents may theoretically have a role in wearing of the floorings: in her study detergents rather postponed than caused erosion of the flooring. This may be a consequence of improved cleaning leading to decreasing chemical attack on the flooring. Great amounts of water may also dilute chemicals, thus decreasing the damaging of the flooring.

In addition to coatings, the chemical resistance of concrete can be affected by its composition and porosity (Shaw 1988, Puumala&Lehtiniemi 1993, Pelletier et al. 2002, Calleja Carrete 2005). The manufacturing technique of concrete, e.g. after-treatment, affects significantly the chemical and mechanical resistance of the flooring (Shaw 1988).

## CHARACTERIZATION OF MATERIALS

Several techniques have been developed over the years to quantify the topography of surfaces. These can broadly be divided into two categories: contact (profilometry) and non-contact methods. In recent decades, a variety of new methods have been developed for the evaluation of surface topography properties, including different microscopic methods e.g. atomic force microscopy, phase shifting interferometry, stereo scanning electron microscopy and laser confocal scanning microscopy. Different techniques used to study surface topography are presented in Table 4.

**Table 4.** Surface topography – a summary of the measurement techniques used for polymeric and ceramic materials (modified from Kuisma 2006, in which the original references are presented)

Device	Resolution		Measurable area	Evaluation of the method	Examples of typical (or possible) materials
	Lateral	Vertical			
Stylus profilometry	100 nm	0.5 nm	Typically a few millimetres	No sample preparation. Stylus can damage the sample. Slow measurement speed in 3D. Not suitable for concrete, similar porous materials or dirty surfaces.	(Plastics)
Optical profiler	350 nm to 9000 nm	0.1 nm	0.2 nm to $10^5$ nm	No sample preparation, non-contacting. Reflective light. Suitable for various surface materials.	Ceramics, polymeric materials
Scanning electron microscopy (SEM)	1 nm to 50 nm (in secondary electron mode)	10 nm to 20 nm	Less than 0.1 mm, up to 10 cm	High magnification imaging. Samples must be vacuum compatible. Requires a conducting surface. Suitable for various surface materials.	Polymeric materials
Atomic force microscopy (AFM)	0.2 nm to 1 nm	<0.03 nm to 0.05 nm	$10^3$ nm to $10^5$ nm	High resolution pictures. Scans small areas, which makes this method unfavourable for the relatively rough surfaces used in animal buildings.	Polymeric materials
Scanning tunnelling microscopy (STM)	0.2 nm	<0.03 nm to 0.05 nm	$10^5$ nm	High resolution pictures. Requires a conducting surface. Scans small areas, which makes this method unfavourable for the relatively rough surfaces used in animal buildings.	Polymeric materials
Confocal microscopy (COM)	500 nm to 4000 nm	2 nm to 2000 nm	100 nm to $6 \times 10^5$ nm	Minimal sample preparation. Background texture often confuses the detectors. Suitable for various surface materials.	Polymeric materials, ceramics

Profilometric analysis is a routine technique used in material science to quantify the morphology of material surfaces or the irregularities of fracture boundaries. Since stylus – material interactions may dramatically affect measurements especially when porous and brittle materials are examined, non-contact techniques offer a better alternative for studies of concrete and similar materials.

Scanning electron microscopy (SEM) allows a qualitative approach to surface topography and is widely used in industrial and biological studies. SEM is a popular technique used in the investigation of structures of surfaces and wear particles. However, interpretation of the images is not necessarily straightforward and does not readily yield quantitative data about the height of surface features.

Atomic force microscopy (AFM) is also known as scanning force microscopy (SFM). The atomic force microscope is a combination of the principles of the scanning tunneling microscope (STM) and stylus profilometer. The atomic force microscope is a versatile tool for measuring surface topography. Because of its wide range of applicability, AFM has become an increasingly important tool for the measurement of surface roughness on the nanometer scale. Additionally, AFM methods are able to measure surfaces in a number of modes: contact, intermittent-contact and non-contact.

Confocal profilers and confocal microscopes have been developed to measure the surface height of smooth to very rough surfaces.

## CONCLUSION

The surfaces in agricultural buildings are subjected to several contaminants. Water and mechanical means (e.g. scrapers and water pressure) are mostly used for cleaning animal houses. The cleanability of floorings and other surfaces in animal buildings can be enhanced with structural solutions (e.g. by using slatted floors) and with coatings or modifications of concrete and other materials. Several methods are available for examining and monitoring cleanliness of surfaces and for their characterization. Methods from other areas of materials sciences have been adopted for use. For cleanability studies, suitable optical, chemical, biochemical and microbiological methods are available for field use. For laboratory studies, the radiochemical measurements offer a potential quantitative alternative. For surface topography studies of relatively rough surfaces such as concrete, scanning electron microscopy, optical profilometer and confocal microscope are among the suitable options. Their area examined is large enough and the porosity (local holes and elevations) does not prevent use of the devices. The principles, operation ranges (e.g. the amount of soil) and suitability for laboratory or field use vary considerably between the methods. Selection of suitable methods is thus needed for laboratory studies and for field use.

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## COMPARATIVE HYGIENE ASSESSMENT OF TECHNOLOGIES FOR ORGANIC MANURE UTILIZATION WITH HIGH CONTENT OF DRY MATTER 1. REDUCTION OF PATHOGENIC MICROORGANISMS IN A CONTINUOUS MESOPHILIC PROCESS OF ANAEROBIC DEGRADATION

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### SUMMARY

The total plate count changes of bacteria (after the introduction of marked strains of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) in a bioreactor, with continuous mesophilic regime of anaerobic degradation of litter substrate from broiler production, is tracked down. Their quantity decrease in the bioreactor is determined, as on the 3<sup>rd</sup> day it is approximately 1 log, on the 5<sup>th</sup> day – 2–4 log, on the 10<sup>th</sup> day – 5–6 log lower compared to the initial quantity. At the end of the process *P. Aeruginosa* is eliminated completely and the other strains remain in low quantities.

**Keywords:** anaerobic degradation, mesophilic regime, marked bacteria

### INTRODUCTION

Searching for efficient methods for decontamination of animal manure and their transformation from waste to useful products is an important ecological task. One of the economically paying possibilities in this respect is methane fermentation with continuous regime. Until now it is still not clear if safe decontamination of the final product is reached after processing of litter from broiler production – afterwards introduced in soil. According to some authors, during manure methane fermentation, a satisfactory decontamination of the incoming animal manure is reached (Baykov et al., 2005). When analyzing the efficiency of the methane fermentation by decontamination of liquid manure, Tivchev et al. (1986) determine considerable decrease of microorganisms' quantity in the final product during mesophile fermentation at a periodic regime. Research of other collectives points that some pathogenic microorganisms remain at a certain extend in the final compost (Petkov and Baykov, 1988; Philipp et al., 2005).

The aim of this research is tracking down the quantitative changes of marked pathogenic microorganisms, introduced into a bioreactor at a continuous mesophilic regime of anaerobic degradation, with a view to assessing the possibilities for decontamination of litter from broiler production in this type of processing and obtaining epizootologically safe final product.



## MATERIAL AND METHODS

*Microorganisms.* Pure cultures of three pathogenic bacterial strains are used in the research: two Gram negative – *Escherichia coli* O6-21C and *Pseudomonas aeruginosa* 33, and one Gram positive – *Staphylococcus aureus* 230. *In vitro* microorganisms show poly-resistance to different antibiotics, mainly to amphenicol antibiotics (Chloramphenicol and Thiamphenicol) and tetracyclines (Tetracycline, Doxycycline and Oxytetracycline).

*Quantitative determination* of microorganisms is carried out using the classical method in serial 10 times increasing dilutions of the analyzed materials in a sterile physiological solution. Cultures are prepared from these dilutions on selected media with and without antibiotics, three for each media and dilution. After incubation at 37°C for 24–48 hours, the average count of the cultivated colonies is determined and the CFU is calculated in 1 ml of the diluted solution.

*Experimental equipment.* The experiments are conducted at a continuous anaerobic degradation process of organic substances in a laboratory bioreactor – with capacity of 2l and working volume 1 l (Simeonov et al., 2006). The anaerobic fermentation is carried out for 22 days at a mesophilic temperature regime  $-34 \pm 0,5^{\circ}\text{C}$ . The bioreactor is charged daily with liquid manure of broiler litter suspension (7% dry matter and pH 6.2); at the same time the same amount of compost is taken out (4.4% dry matter and pH 7.2).

After preliminary determination of bacterial quantity of *E. coli*, *Pseudomonas spp.* and *Staphylococcus spp.* and the total plate count of the incoming and the outgoing material, the three marked microbial strains are introduced in the bioreactor each in concentration  $10^7$  CFU/ml from its total quantity. Samples from the outlet (the ready compost) are taken on the 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup>, 14<sup>th</sup> and the 21<sup>st</sup> day for determination of the quantity of marked and unmarked microorganisms.

The statistical analysis of the results is done using one-way analysis of variance (ANOVA) and Dunnett post-hoc test.

## RESULTS AND DISCUSSION

The results from the analysis are presented in table 1.

As it is seen from the summarized data, during the first week after the introduction of marked bacteria, a gradual growth of the total plate count of microorganisms in the bioreactor is observed., which is statistically reliable on the 7<sup>th</sup> day ( $P < 0.05$ ). This probably is due to the introduction of these bacteria into the system, as well as the every day addition of liquid manure, which contains microorganisms, too. From the 10<sup>th</sup> day on, a gradual and reliable decrease of the total plate count of bacteria is determined with more than 1 log compared to the initial quantity and to the previous periods of analysis ( $P < 0.05$ ). These results correspond to the obtained by Petkov and Baykov (1988) at similar conditions of fermentation.

Analogical results are observed with the total plate count of *E. coli*, *Pseudomonas* bacteria and staphylococcus bacteria. After reliable increase on the 3<sup>rd</sup> day, due to the introduction of marked microorganisms ( $P < 0.001$ ), a gradual decrease of total plate count of the corresponding bacteria as well as of the marked strains begins, which can be observed in table 1. This decrease is expressed the highest on the 5<sup>th</sup> day – with approximately 2–4 log, and in the middle of the experiment (on the 10<sup>th</sup> day), the quantity of microorganisms reaches its minimal values. Factors, further this, could be the every day taking out of compost (together with bacteria), the unfavorable conditions in the bioreactor and the competition among microorganisms.

**Table 1.** Dynamics of microorganisms in a bioreactor at a continuous process

Sample	Total plate count bacteria	<i>E. coli</i> – total plate count	<i>Pseudomonas spp.</i> – total plate count	<i>Staphylococcus spp.</i> – total plate count	<i>E. coli</i> marked	<i>P.aeruginosa</i> – marked	<i>S. aureus</i> marked
Inlet before addition of marked bacteria	3,4.10 <sup>9</sup> * ±0,38**	8,0.10 <sup>4</sup> ±0,37	1,7.10 <sup>4</sup> ±0,38	2,7.10 <sup>5</sup> ±0,87	–	–	–
Outlet before addition of marked bacteria	3,3.10 <sup>9</sup> ±0,35	2,1.10 <sup>4</sup> ±0,54	1,3.10 <sup>4</sup> ±0,26	2,2.10 <sup>5</sup> ±0,73	–	–	–
3 <sup>rd</sup> day	4,8.10 <sup>9</sup> ±0,9	3,6.10 <sup>6</sup> ±0,71	1,4.10 <sup>6</sup> ±0,58	1,5.10 <sup>6</sup> ±0,88	3,1.10 <sup>6</sup> ±0,62	1,2.10 <sup>6</sup> ±0,39	1,4.10 <sup>7</sup> ±0,58
5 <sup>th</sup> day	5,6.10 <sup>9</sup> ±0,78	1,2.10 <sup>6</sup> ±0,45	7,9.10 <sup>3</sup> ±0,87	2,6.10 <sup>5</sup> ±0,87	1,2.10 <sup>3</sup> ±0,55	3,2.10 <sup>3</sup> ±0,43	3,6.10 <sup>3</sup> ±0,49
7 <sup>th</sup> day	6,9.10 <sup>9</sup> ±0,79	1,8.10 <sup>6</sup> ±0,52	3,9.10 <sup>3</sup> ±0,65	2,1.10 <sup>4</sup> ±0,89	1,5.10 <sup>3</sup> ±0,68	0,96.10 <sup>2</sup> ±0,33	1,3.10 <sup>2</sup> ±0,46
10 <sup>th</sup> day	3,9.10 <sup>9</sup> ±0,52	7,7.10 <sup>5</sup> ±0,77	1,6.10 <sup>3</sup> ±0,56	4,9.10 <sup>3</sup> ±0,98	0,5.10 <sup>3</sup> ±0,18	0,27.10 <sup>2</sup> ±0,06	0,12.10 <sup>2</sup> ±0,003
14 <sup>th</sup> day	2,1.10 <sup>9</sup> ±0,76	1,7.10 <sup>5</sup> ±0,68	6,4.10 <sup>4</sup> ±0,98	4,6.10 <sup>4</sup> ±0,79	2,7.10 <sup>3</sup> ±0,48	2,4.10 <sup>3</sup> ±0,88	6,0.10 <sup>2</sup> ±0,38
21 <sup>st</sup> day	1,2.10 <sup>8</sup> ±0,32	7,6.10 <sup>4</sup> ±0,78	5,0.10 <sup>4</sup> ±0,53	3,5.10 <sup>4</sup> ±0,86	5,9.10 <sup>3</sup> ±0,78	–	1,0.10 <sup>3</sup> ±0,45

\* Average. \*\* Standard deviation

However after that an increase begins, probably due to growth in the bioreactor, which is not statistically reliable towards the two previous measurements ( $P > 0.05$ ). This increase is possible at the corresponding conditions, due to the fact that the analyzed microorganisms are facultative anaerobes; they also are characterized with high resistance to unfavorable physical and chemical factors. It is interesting that on the 10<sup>th</sup> day only the total plate count of *E. coli* is increased in comparison with all others ( $P < 0.001$ ), after that day it starts to decrease while for the others a slight increase is observed. Obviously at this stage the bacterial balance in the system is disturbed in favour of *E. coli* which dominates, suppressing other bacteria – this probably is due to the antibiotics that this microorganism excretes – colicines. Its dominance to other tracked species is kept till the end of the experiment.

The behavior of the marked strain of *P. aeruginosa* is interesting. Its quantity oscillates during the second week, on the 10<sup>th</sup> day it rapidly decreases and after a slight increase on the 14<sup>th</sup> day, at the end of the experiment it is eliminated completely. At the same time the left saprophytes, *Pseudomonas spp.*, normally present in liquid manure, are in relatively high amount. Maybe it has something to do with interspecies antagonism in the genus, probably through bacteriocines. It is less likely to be a display of sensitivity to colicines, excreted by the dominating specie *E. coli* in the system, because obviously the other species from the *Pseudomonas* are not influenced much from their activity. On the other hand the slight increase of *S. aureus* at the end could be related to vanishing of *P. aeruginosa*.

There is data that at thermophilic anaerobic fermentation, the decrease of pathogenic microorganisms is considerable in comparison to that at mesophilic regime (Philipp et al., 2005;

Sahlström et al., 2005). After termination of the thermophilic process the authors do not determine presence of pathogenic microorganisms.

From the obtained results it is obvious that the quantity of the introduced pathogenic microorganisms considerably decreases and reaches minimal values, but they are not eliminated completely except for *P. aeruginosa*. Even though that their quantity in the end of the continuous mesophilic methane fermentation is very low, there is certain risk transferring these microorganisms to the environment through compost. When mixing them with soil however, their quantity per unit area will be neglectfully low, so the risk for animal infection would be minimal.

## CONCLUSIONS

At continuous mesophilic regime of anaerobic degradation, for 21 days, a high level of microbial decontamination is reached regarding pathogenic microorganisms. Using this type of fermentation does not provide complete decontamination of manure.

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## COMPARATIVE INVESTIGATION OF ALTERNATIVE METHODS IN DISINFECTANT TESTING ACCORDING TO THE DVG-DISINFECTANT TESTING GUIDELINES (GERMANY)

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### SUMMARY

In the course of international standardization of disinfectant testing, the existing national testing directives have to be adapted or recreated. Thereby, the current methods of disinfectant testing are critically assessed. Special attention is paid to find possible alternatives to the expensive dilution methods in tubes.

Therefore, two alternative methods of qualitative suspension tests were compared in this study. The current procedure using 10 millilitre test tubes was compared to an alternative microdilution method in microtiter plates. The goal of this work was to evaluate the practicability of these two methods in a suspension test for bactericidy and fungicidy, as well as comparing of the repeatability of these methods. Moreover, the selection of the proposed alternative and additional micro-organisms were tested for their suitability and possible advantages.

In accordance to the above stated purposes, the work focused on disinfectant testing with bacteria and fungi which are relevant in animal housing. A total of four disinfectants, belonging to different chemical groups, were included.

When compared with the method in tubes the microdilution method showed advantages as it saves time and material, and it increases the number of possible realisable assays.

The analysis of the repeatability of both investigated methods of the qualitative suspension test showed that the rate of differences between the attempts was at the microdilution method 39.7% and 45.3% respectively. These differences were determined at the tube method 45.3% and 49.8% respectively. The distribution of these internal differences of both methods lay within a comparable range. The mean coefficient of inter assay variation of the tube method was 39.5%, whereas the mean coefficient of variation of the microdilution method was slightly higher with 43.2%. Only 25.8% of the determined effective disinfectant dilutions differed in the parallel accomplished direct comparison. 95.7% of differences differed in only one dilution step and were, thus, regarded as not significant. However, in 80.6% of the differences, the tube method required a higher concentration of the disinfectant for killing the test organisms. Because of these unequal distributions, it was not possible to statistically prove the equality of both methods.

In summary, the results of this study show that the alternative microdilution method appears to be as repeatable as the so far used tube method. Especially the mentioned advantages in practicability and material effort make the microdilution method to a serious alternative to the current tube method.

However, the results of the disinfectant testing with four disinfectants showed that the repeatability still present a difficulty in disinfectant testing. Missing analogy in 44.4% of the duplicates demonstrates this assumption.

## BIOFILTRATION OF VOLATILE INORGANIC COMPOUNDS IN THE HATCHERY

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### ABSTRACT

The studies on the treatment of air vented from the hatchery hall were performed with a prototype enclosed biofilter.

The biofilter was fitted to the ventilating duct outlet of the room with 8 hatchers (AS-4H, Petersime, Zulte, Belgium) and 12 incubators (AS-4S, Petersime, Zulte, Belgium) with the input of 115 eggs. The biofilter of 2.0 x 1.8 x 1.8 m dimensions was composed of the following components: high pressure fan of 1500m<sup>3</sup>/h maximal capacity, air humidifier and biofiltration chamber. This chamber was divided into three independent parts to facilitate the simultaneous assessment of the biofiltration properties of three different fillings – beds. In the present investigation, the following media were used: organic and organic – mineral. Removal efficiency of inorganic pollutants in the vented air was estimated by the ion chromatography method.

The performed research focused on the evaluation of control performance of air vented from the chicken hatchery room on the organic and mineral organic media in the enclosed container biofilter. The investigation proved very high treatment efficiency of the biofilter fitted at the ventilation system outlet, irrespective of a filter material applied. Reduction of total inorganic compounds reached nearly 40%, high in the case of ammonia and nitrite (nearly 50%), while low for nitrate (–73%). The best treatment properties (retaining) for air pollutants were recorded for the bed with bentonite.

**Keywords:** inorganic compounds, hatchery, biofiltration

### OBJECTIVE

Animal production, in that poultry farms, also constitutes a source generating a broad range of chemical substances such as ammonia, hydrogen sulfide, mercaptans, indol, skatole, phenol as well as aldehydes, ketones, alcohols – the compounds showing the odorforming, toxic or even carcinogenous properties [Tymczyna & et. al., 2004a; 2004b]. Some of them undergo complex transformations in the air that can enhance their harmfulness.

Specificity of the off-gases produced at the animal breeding imposes the application of the methods that prove efficient towards such a broad spectrum of contaminants and the biotreatment methods for gas pollutants control satisfy this requirement. The biofiltration process is performed in so-called biofilters where the pollutants exposed to the direct contact with a bacteria population that naturally colonizes the filter material like, soil, peat compost or is deliberately introduced, are partly or completely degraded [Ramirez-Lopez et al., 2003].

The objective of the present work was to evaluate biotreatment performance of air contaminated with inorganic pollutants vented from the chicken hatchery hall during the biofiltration process.

## METHODS

The study was conducted at the Poultry Hatchery in Dębówka, 20km south of Warsaw, Poland. The hatchery with annual output of 20 to 25 million Cobb and Ross meat hens, which represents 4% of the national production.

A bio-filter was installed in the ventilation outlet of the hatching room, which was equipped with 8 hatchers (AS-4H, Petersime, Zulte, Belgium) and 12 incubators (AS-4S, Petersime, Zulte, Belgium) with an input of 115th eggs. The bio-filter measured 2.0 x 1.8 x 1.8 meters, and included the following components: a high pressure fan with a maximum capacity of 1500 m<sup>3</sup>/h; an air humidifier; and a bio-filtration chamber (constructed by the present authors).

The bio-filtration chamber was divided into three independent parts to facilitate the simultaneous assessment of bio-filtration properties of three different fillings – beds. The depth of the filter medium was between 1.2 and 1.4meters.

In this study, the following media were used: organic medium containing 50% compost and 50% peat – OM; organic-mineral medium containing 20% bentonite, 40% compost and 40% peat – BM; organic-mineral medium containing 20% halloysite, 40% compost and 40% peat – HM.

Six series of experiments were carried out during the 10mo course of the study. In each series of experiments, 10 air samples were collected: 4 in the air intake duct of the bio-filter – in the hatchery room, and 6 at the air outlet duct, i.e. 2 at each bio-filtration chamber.

The determinations of volatile inorganic compounds in the samples drawn into the sparger washers were performed in compliance with the Polish standards for ion chromatography using the liquid chromatograph Waters produce linked with Analytical Column IC-PAK Anion HR filled with Waters anion solvent combined with conductometer detector and UV.

The following statistical parameters were calculated on the basis of all of the research results: number of observations, arithmetic mean±standard deviation, arithmetic mean error and coefficient of variation. On the grounds of mean concentrations of the pollutants prior to and after the biofiltration application, a mean reduction rate of the pollutants could be calculated. Open biofilter removal efficiency was characterized by % reduction.

The mean levels of volatile inorganic compounds in the hatching room air were compared with the mean contamination levels in the air after bio-treatment using the Tukey's and Dunett's tests. The sampling site-specific coefficients for filter media efficiency were calculated using the Kruskal-Wallis nonparametric tests. The calculations were performed with SAS v. 9 and Statistica v. 6.0 software packages application.

## RESULTS

Among the inorganic pollutants determined in the hatchery room, the presence of ammonia, nitrates, nitrites, chlorides, sulfides (Tab.1) was confirmed. The highest content was reported for ammonia (0,49 mg/m<sup>3</sup>), while a concentration of other volatile pollutants appeared to be low, often at ion chromatography detection threshold (0.01 mg/m<sup>3</sup>)

In the present research, there was observed a decrease of the inorganic compounds level after the biotreatment completion (Tab.1). The highest differences were found for the ammonia concentration whose content showed a clear decline in the air samples collected after the biofilter. Only the nitrates concentration in the air leaving the biofilter slightly increased on the organic bed (OM) and mineral with halloysite additive. The present study revealed a decreased content of the inorganic compounds after the biotreatment process (Table 1), the highest differences were noted in the ammonia concentration which exhibited a substantial fall in the air samples taken after the biofilter. However, only a nitrates level in the air leaving the biofilter was slightly elevated on the organic medium (OM) and mineral with a halloysite component (HM). The statistical analysis made with Tukey and Dunett tests did not reveal any significant differences between the concentration of the compounds recorded in the hall and their level after the biotreatment process completion.

**Table 1.** Inorganic compound concentration before and after biotreatment (mg/m<sup>3</sup>)

COMPOUND TYPE			BEFORE	AFTER		
			hatchery hall	OM	HM	BM
Total	N		22	12	13	13
	M±SD		0,79±0,77a	0,55±0,66a	0,54±0,4 a	0,34±0,13 a
	min.		0,2	0,2	0,2	0,2
	max.		3	2,6	1,5	0,6
Identified:	ammonia	M±SD	0,49±0,81a	0,31±0,6 a	0,32±0,42 a	0,15±0,07 a
		Min.	0,1	0,1	0,1	0,1
		Max.	3	2,2	1,3	0,3
	nitrates	M±SD	0,01±0,04 a	0,03±0,12 a	0,02±0,06 a	0,00±0,0 a
		Min.	0	0	0	0
		Max.	0,2	0,4	0,2	0
	nitrites	M±SD	0,02±0,05 a	0,00±0,0 a	0,03±0,05 a	0,00±0,0 a
		Min.	0	0	0	0
		Max.	0,2	0	0,1	0
	chlorides	M±SD	0,19±0,15 a	0,14±0,13 a	0,12±0,12 a	0,13±0,14 a
		Min.	0	0	0	0
		Max.	0,6	0,4	0,4	0,4
	sulphates	M±SD	0,07±0,05 a	0,07±0,05 a	0,06±0,05 a	0,06±0,05 a
		Min.	0	0	0	0
		Max.	0,1	0,1	0,1	0,1

a,b.. – values in columns denoted with different letters differ significantly at  $p \leq 0,05$  Tukey's and Dunett's tests

N – number of samples analyzed statistically

M±SD – arithmetic mean ± standard deviation

On the basis of the compared concentrations for each filter material, there was calculated its treatment performance (Table 2). The mean reduction percentage of all the identified inorganic compounds fluctuated within a broad range, i.e. from minimum minus 600% up to maximum – 100%. The highest treatment efficiency was recorded for nitrites (53,7%) while the lowest for nitrates (–73,7%). The nitrites removal rate appeared to differ statistically significantly ( $P \leq 0,05$ ), subject to a medium investigated. The elimination level of the inorganic pollutants

averaged 39,8%, whereas a mean removal rate of all the identified inorganic substances showed considerable fluctuations from -600% to 100%.

**Table 2.** Percentage of inorganic compounds reduction [%]

COMPOUND TYPE	Mean			MB			MH			MO			
	M±SD	min	max	M±SD	min	max	M±SD	min	max	M±SD	min	max	
Total	38,6±15,8	-600	91	57,0±36,3	-16,7	91	31,5±99,7	-216,7	68,5	30,1±25,9	-600	88,8	
Identified	ammonia	48±13,4	-500	90,5	70,2±38,5	-16,7	90,5	35,8±84,6	-183,3	66,7	37,2±21,9	-500	88,2
	nitrates	-73,7±53	-131	25,2	100±0	100	100	-69,2±31	-98,4	-38,7	-266,7±219	-500,5	100
	nitrites	53,7±86,8*	-100	100	100±0	100	100	-35,4±77	-100	33,3	100±0	100	100
	chlorides	32,5±37,5	-7,7	100	31,5±42,9	-7,7	100	39,6±37,3	7,7	100	25,8±41,6	-7,7	100
	sulphates	13,2±26	-16	34,2	15,4±8,6	8,4	25	15,4±8,6	8,4	25	8,3±10,5	-3,5	15,9

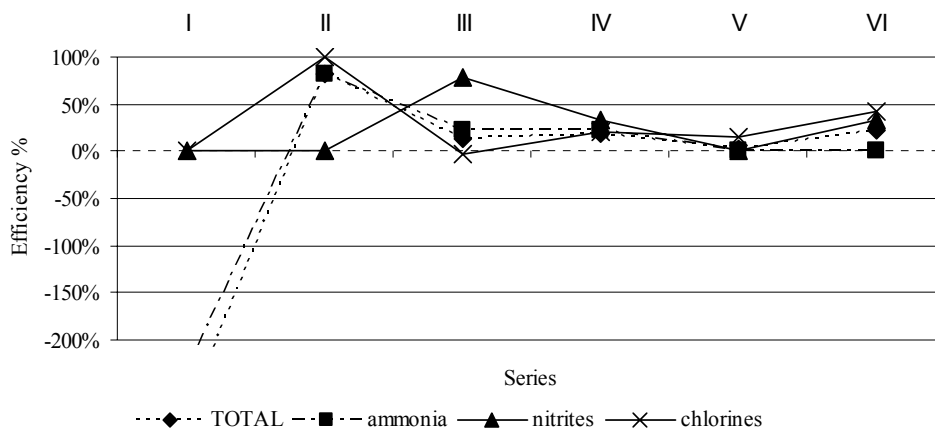
\* statistically significant differences at  $p < 0,05$  Kruskal-Wallis tests

M±SD – arithmetic mean ± standard deviation

Regarding all the tested media, the most beneficial properties for inorganic compounds bioreduction were shown for the bentonite supplemented medium (BM) (Table 2). This medium was characterized by the highest performance of a chloride removal rate, yet very low for nitrites and nitrates with negative values obtained. The lowest treatment efficiency was recorded for nitrites (-266,7%) in the organic bed (OM).

Degradation of the inorganic substances throughout the research period demonstrated a high variation (Fig.1). In I research series, the negative values for the inorganic substances (total) and ammonia contents were obtained that indicated a substantially higher number of these compounds after biotreatment than in the hatchery hall. However, the continued operation of the biofilter brought increased efficiency of biological treatment as in II research series the highest values for chlorides removal (100%) and ammonia reduction (83%) were determined. The nitrites decomposition, though, appeared to be some different as their highest elimination rate was established in 30wk of the biofilter work, that is III research period. The ammonia reduction level gradually declined in II research period, i.e from 10 wk of biofilter work till the experiment completion.





**Figure 1.** Percentage of inorganic compounds reduction at each series

## CONCLUSIONS

The available Polish and foreign literature presents the simulation investigations of single compound degradation at the laboratory-scale [Classen et al.,2000]. However, the literature reviews only few studies on the biological waste gas treatment under the production conditions, where the generated volatile pollutants are not homogeneous but constitute a mixture of different chemical compounds. The present work, though, is one of these attempts concerning the investigations undertaken in the real conditions. Despite a fact that the studies revealed very high variation of the VOC bioreduction which did not allow choosing statistically confirmed most beneficial medium, yet a following conclusion can be drawn. A bentonite supplemented filter material (20%) can seriously decrease pollutants amount released from this type of a contamination source, in particular ammonia and its degradation products.

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## INVESTIGATION OF ENVIRONMENTAL POLLUTION IN INDUSTRIALLY CONTAMINATED AREA WITH EXTENSIVE ANIMAL PRODUCTION

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### SUMMARY

We examined quality of potable water in 20 wells, 3.5 to 14 m deep, of varying capacity in a village in industrially contaminated area with extensive animal production. Our investigations focused on common chemical and bacteriological indicators of contamination. In addition to that 71 elements were determined by the AAS method. The limits for heavy metals were exceeded in three wells (Ni in one, Sb in two and As in one). One or two chemical indicators of pollution were exceeded 10 wells, 3 in two well and 5 in one well. None of the wells could be considered completely safe from the bacteriological point of view.

**Keywords:** drinking water, environmental pollution, heavy metals, bacteriological quality

### INTRODUCTION

Almost every country in the world faces problems with the quality of drinking water. Sources of drinking water are polluted by various human activities in many different ways. Some pollutants cause immediate problems but some can be seen only after longer period. Contamination of atmosphere results in accumulation of metals and toxic chemicals in the soil from which they can be leached to ground and surface water, particularly in regions exposed to acidic rains. Animal production and human wastes can contaminate water with pathogenic bacteria, viruses and protozoa and some macronutrients. Buildup of chemicals in organisms and a food chain may impair different processes in the body and contribute to cancer. In advanced countries the sources of drinking water are protected and those intended for mass consumption are frequently checked and, if necessary, treated (Ondrašovič et al., 1997). However, considerable number of people is still depended on individual sources the quality of which may be questionable and is not protected by regular disinfection.

The aim of the present study was to investigate the quality/pollution of individual water sources in an industrially polluted area with some extensive animal production and determine the safety of water used for drinking and preparation of food.

### MATERIAL AND METHODS

Our investigations were carried out in the period of one year in a village (approximately 500 inhabitants) with extensive animal production, located in environmentally polluted region affected negatively by mining activities and processing of complex Fe and Cu ores. We examined water in 20 wells, 3.5 to 14 m deep, of varying capacity. The village is not connected to mass drinking water supply.

Our investigations were divided to chemical and microbiological part. The chemical examination included determination of pH, ammonium ( $\text{NH}_4^+$ ), nitrites ( $\text{NO}_2^-$ ), nitrates ( $\text{NO}_3^-$ ), chlorides ( $\text{Cl}^-$ ), phosphates ( $\text{PO}_4^{3-}$ ), free chlorine ( $\text{Cl}_2$ ), chemical oxygen demand ( $\text{COD}_{\text{Mn}}$ ) and additional 71 different elements including heavy metals. Spectrophotometric methods were used for determination of ammonium, nitrites and phosphates (Nessler reagent; sulphanilic acid and N-(1-naphthyl)-ethylenediamine dihydrochloride; ammonium molybdate, ascorbic acid, antimony potassium tartrate). Nitrates were determined by ion selective electrode (ORION Research), chlorides and free chlorine by titration (argentometric and iodometric, resp.) and chemical oxygen demand by boiling with potassium permanganate for 10 min. In addition to these determinations 71 elements were determined twice by the AAS method.

Microbiological examination included determination of colony counts at 22°C and 37°C on meat-peptone agar, coliform bacteria and *E. coli* on Endo agar at 37° and 43°C with confirmation by lactose fermentation.

## RESULTS AND DISCUSSION

Results of AAS examination for some metals for which the respective national standard (Statutory order of SR No. 354/2006 of the Civil Code) has set maximum contaminant levels (MCL) are presented in Table 1. The results show that MCL were exceeded in three wells. Higher concentration of nickel ( $27 \mu\text{g.l}^{-1}$ ) was detected in Well 6, of antimony in Well 16 ( $12 \mu\text{g.l}^{-1}$ ) and of both antimony and arsenic in well 18 (8.6 and  $19.0 \mu\text{g.l}^{-1}$ , resp.). However, considerable variations were observed between the wells. MCL for other metals of interest were not exceeded (Se, Ag). From among other metals for which limits are specified in the respective regulation (Mn, Al, Fe, Cu, Zn and Na only the acceptable level of iron ( $0.2 \text{ mg.l}^{-1}$ ) was exceeded in 15 wells. The highest concentration of Fe in Well 4 reached  $0.460 \text{ mg.l}^{-1}$ .

**Tab. 1** Level of selected metals determined by the AAS method

Well No.	Sb	As	Cr	Cd	Ni	Pb	Hg
	MCL ( $\mu\text{g.l}^{-1}$ )						
	5	10	50	3	20	10	1
1	0.52–0.72	0.70–0.81	0.05–0.53	0.130–0.080	11–12	0.05–0.06	<0.006–0.007
2	0.52–0.68	0.55–0.62	0.30–0.42	<0.018–0.018	7.8–8.6	0.03–0.05	<0.006–0.006
3	1.80–2.30	0.71–0.85	0.28–1.00	<0.018	13–15	0.01–0.04	<0.006–0.007
4	0.43–0.80	0.71–1.20	3.30–5.70	<0.018–0.018	3.7–5.3	0.06–0.17	0.010–0.03
5	0.41–0.61	0.45–0.81	0.18–1.30	0.056–0.063	8.2–12	0.09–0.14	<0.006–0.007
6	0.79–1.80	1.10–1.90	0.05–2.50	<0.018–0.018	16–27	0.03–0.04	<0.006
7	1.40–2.50	0.30–1.20	0.18–0.57	0.075–0.082	5.8–6.8	0.02–0.04	<0.006
8	0.35–1.10	0.62–1.50	3.30–5.60	<0.018–0.026	2.9–3.3	0.03	<0.006–0.03
9	0.20–1.20	0.39–0.77	1.60–1.80	0.018–0.030	7.9–8.0	0.03	<0.006–0.006
10	0.10–0.17	0.93–1.40	11.0–16.0	<0.018–0.018	1.7–2.6	0.02–0.04	0.007–0.008
11	0.11–0.25	1.90–2.00	6.30–4.80	<0.018–0.037	2.0–6.6	0.02–0.03	<0.006–0.006
12	0.27–2.00	0.25–0.58	0.34–0.72	0.026–0.048	1.2–1.4	0.03–0.16	<0.006–0.007
13	2.00–1.00	1.10–1.10	0.15–1.00	<0.018–0.018	1.3–2.7	0.07–0.13	<0.006–0.010
14	1.20–2.60	0.82–1.50	0.11–0.45	0.018–0.063	1.3–3.1	0.06–0.43	<0.006
15	0.48–1.70	0.35–0.84	0.18–0.31	<0.018–0.026	0.9–3.4	0.02–0.09	<0.006

**Table 1.** Continuation

Well No.	Sb	As	Cr	Cd	Ni	Pb	Hg
	MCL ( $\mu\text{g}\cdot\text{l}^{-1}$ )						
	5	10	50	3	20	10	1
16	2.90–12.0	1.50–5.80	0.10–0.37	<0.018	0.6–3.2	0.02	<0.006
17	3.10–4.10	1.90–5.00	0.14–0.72	<0.018–0.018	1.2–4.3	0.03–0.06	<0.006
18	2.10–8.60	4.20–19.0	0.12–0.54	<0.018	1.3–3.1	0.02	<0.006
19	1.50–2.40	1.50–3.00	0.13–0.67	<0.018	1.2–2.4	0.02–0.06	<0.006–0.006
20	1.30–0.95	1.70–1.10	0.78–0.59	0.018–0.026	1.0–1.2	0.02	<0.006–0.008

MCL – maximum contaminant level

Metals are inorganic substances that occur naturally in geological formations. Some are essential for life and are naturally available in our food and water. In addition to them, drinking water may contain metals which cause chronic and acute poisoning. Contamination of water resources by poisonous metals occurs largely through human activity. These activities include industrial processes, such as mining and processing of metal ores, agricultural activities, discarding of wastes in landfills (ALLOWAY, AYRES, 1993).

Arsenic in drinking water causes bladder, lung and skin cancer, and may cause kidney and liver cancer. Arsenic harms the central and peripheral nervous systems as well as heart and blood vessels, and causes serious skin problems. It also may cause birth defects and reproductive problems (NRDC, 2001).

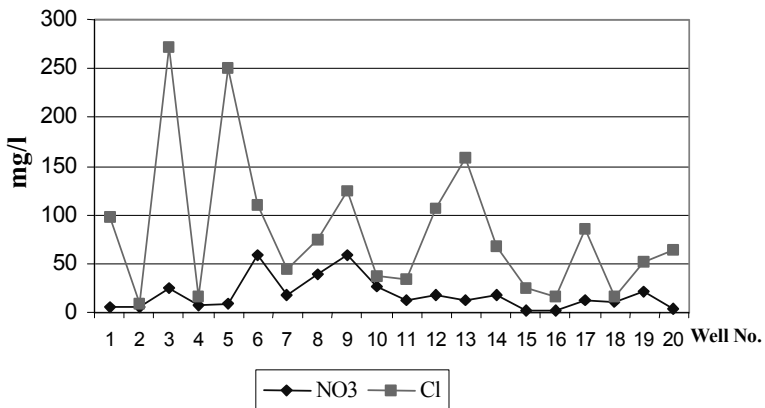
We have become interested in the concentration of antimony in drinking water since 1998, on the basis of WHO recommendation, particularly with respect to its carcinogenic effects. Continuous exposure to antimony may result in lung diseases, heart problems, diarrhoea, vomiting and stomach ulcers (ATSDR, 1992).

Nickel concentrations in groundwater depend on the soil use, pH, and depth of sampling. The average concentration in groundwater in the Netherlands ranges from  $7.9 \mu\text{g}\cdot\text{l}^{-1}$  (urban areas) to  $16.6 \mu\text{g}\cdot\text{l}^{-1}$  (rural areas). Acid rain increases the mobility of nickel in the soil and thus might increase nickel concentration in groundwater (ICPS, 1991). In groundwater with a pH below 6.2, Ni concentrations up to  $980 \mu\text{g}\cdot\text{l}^{-1}$  have been measured (RIVM, 1994). Allergic contact dermatitis is the most prevalent effect of nickel in the general population. Soluble Ni exposure increased risk of cancer.

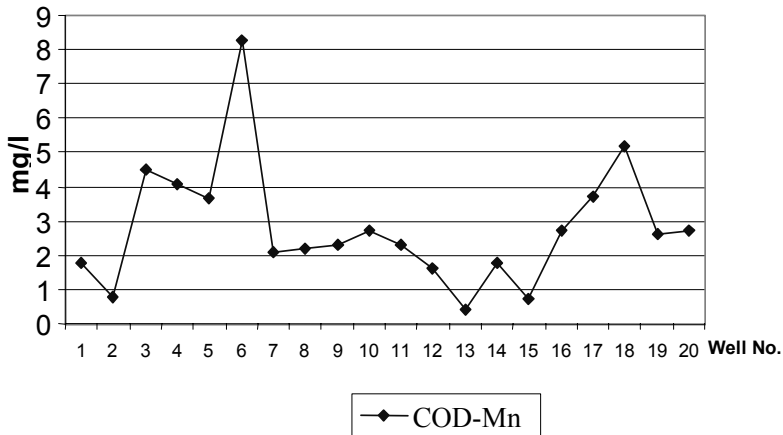
Our chemical examination focused on inorganic indicators of contamination of water with wastes (pH, ammonium, nitrites, nitrates, chlorides, phosphates and chemical oxygen demand), and some efforts to alleviate the potential consequences of such contamination (free chlorine).

The pH value in well ranged from 6.03 to 7.68 so there is no indication of excessive acidification in the area. Of all remaining chemical parameters determined in water ammonia, phosphates and free chlorine were exceeded each only in one well at one sampling. Nitrites exceeded the acceptable level ( $0.1 \text{ mg}\cdot\text{l}^{-1}$ ) in three wells, in each only in one sample. Out of 20 wells only in 7 none of the examined parameters were exceeded but one of these 7 wells showed increased concentration of antimony. Mean levels of nitrates, chlorides and chemical oxygen demand for the period of examination are shown in Fig. 1 and 2. The acceptable limit for nitrates in drinking water is  $50 \text{ mg}\cdot\text{l}^{-1}$ , for chlorides  $100 \text{ mg}\cdot\text{l}^{-1}$  and for  $\text{COD}_{\text{Mn}}$   $3 \text{ mg}\cdot\text{l}^{-1}$ . On the basis of chemical examination Well No. 6 appeared to be most contaminated as in this well 5 of the determined chemical parameters were exceeded.

Determination of plate counts of selected micro-organisms revealed that total coliforms were present in all wells (in 10 ml volume) and in every one of them *E.coli* were detected at least at one sampling. This indicates that none of the wells could be considered safe from the bacteriological point of view. Moreover, in 8 wells we detected free chlorine, in one (No.7) above the permissible level ( $0.3 \text{ mg.l}^{-1}$ ), which suggested an individual, although unsuccessful effort to make the water safer by chlorination.



**Figure 1.** Mean concentration of nitrites and chlorides in examined well



**Figure 2.** Mean values of  $\text{COD}_{\text{Mn}}$

Agricultural production in the respective area resulted obviously in general contamination of soil and groundwater. Although the number of families keeping some farm animals has decreased recently, pigs and cattle are still kept and their manure is applied to gardens and adjoining fields. Moreover, the rules for protection of individual sources of drinking water (STN 75 5111, 1993) have not been observed which contributes to the unfavourable situation.

Similar situation was reported in the study conducted by da Silva Alberto (2002), who observed similar situation in another location in SR and observed relationship between precipitations and plate counts of coliform bacteria and E.coli in individual sources of drinking water.

## CONCLUSION

Results of examination of individual water sources in industrially contaminated area with extensive animal production showed that the levels of heavy metals were exceeded only sporadically only in three wells and the maximum values were not even twofold of the maximum contaminant level except for Sb on one occasion (2.5-fold). However, the general contamination of the area due to extensive animal production and failure to comply with rules for protection of water sources caused that none of the wells was completely safe from the microbiological point of view.

## ACKNOWLEDGEMENT

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## BIODIVERSITY AND CONCENTRATION OF AIRBORNE FUNGI IN CHICKEN HOUSE

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### ABSTRACT

The biodiversity and concentration of airborne fungi in chicken house were investigated. The air samples were collected by means of 6-stage Andersen sampler and common fungal counting media. 78 fungal species were identified from air samplings after mycological examination. They are mainly mitosporic fungi. *Cladosporium*, *Penicillium*, *Aspergillus*, *Candida*, *Alternaria* and *Fusarium* were the predominant fungal genera, including ten species of avian pathogenic fungi, such as *A. flavus*, *A. fumigatus*, *A. niger* and *F. sporotrichioides* et al. The average concentration of airborne fungi in the chicken house was  $2.3 \times 10^3$  CFU/m<sup>3</sup>. The concentrations of *A. flavus*, *A. fumigatus* and *A. niger* were relatively higher in the air of chicken house. The distribution of airborne fungi in the chicken house shows normal logarithm, the spores of *Cladosporium*, *Penicillium* and *Aspergillus* were mainly distributed within C and D stages (3.0–6.0 μm), while stage A and B (>6.0 μm) as well as E and F stage (<3.0 μm) relatively less. On the other hand, *C. albicans*, *Histoplasma capsulatum*, *Cryptococcus neoformans* and *Coccidioides immitis*, which occur uncommonly but pose potential threat to public health. The result provides alerting data for controlling the avian mycosis and scientific basis for making preventive and cure measures.

The sweeping of avian flu across Asia has given a warning to human being that we should give more attention to the threat. In recent years, the widespread application of broad spectrum antibiotics has resulted in the escalation of avian diseases caused by fungi, in which, mortality rate caused by aspergillosis might reach 24–94.5%. The researchers have found that *Aspergillus* species are predominant pathogens in chicken houses. Pinello et al (1977) have isolated 73 species of fungi from the chicken house air, the feed, the pad grass and chicken bodies, but the type and the density of active fungal spores (specially pathogenic fungi) inhaled by animals in the air are the key aspects which induce respiratory tract mycosis in human beings and poultry. By far there have been fewer reports on airborne fungi in chicken house.

This article takes the airborne fungi in chicken house as a factor to evaluate their harm on occupation for the first time. We have conducted systematic classification of airborne fungi in chicken house, in order to provide a scientific base for the early warning and controlling methods of avian diseases caused by fungi.

## 1. MATERIAL AND METHOD

### 1.1 Air sampler

The air samples were collected by means of 6-stage Andersen sampler(Liaoyang Application Technical Research Institute, China), its effective current interception diameter from A to F level are 8.2 $\mu$ m, 6.0 $\mu$ m, 3.0 $\mu$ m, 2.0 $\mu$ m, 1.0  $\mu$ m and 0.65 $\mu$ m in turn. and common fungal counting media

### 1.2 Fungal isolation and identification

Rose Bengal Chloromycin agar (RBC) was used as the medium for the isolation of airborne fungi in chicken house. After indoor incubation, all fungal colonies were counted and purified. For accurate morphological identification of the fungal species, various media like malt extract agar (MEA), czapek yeast agar (CYA), potato dextrose agar (PDA), potato sucrose agar (PSA), Nirenberg sucrose agar (SNA) were used to grow the fungi.

## 2. RESULTS AND ANALYSIS

### 2.1 Composition and concentration of airborne fungi in chicken house

4,709 fungal colonies were obtained from 108 air samples in chicken houses. 78 fungal species were morphologically identified, including 442 isolates of yeasts and yeast-like fungi belonging to 8 species of 6 genera, 4,277 isolates of mitosporic fungi belonging to 67 species of 29 genera, 4 isolates of ascomycetes belonging to one species of one genus, and seven isolates of zygomycetes belonging to two species of two genera. Moreover, 432 isolates of 33 different colonies were not identified because of their non-sporulation. The average concentration of airborne fungi in the chicken house is  $2.3 \times 10^3$  CFU/ m<sup>3</sup>, in which the concentration of mitosporic fungi is  $2.1 \times 10^3$  CFU/ m<sup>3</sup>, accounting for 90.8%, the concentration of yeast and yeast-like fungi  $2.2 \times 10^2$  CFU/ m<sup>3</sup>, accounting for 9.4%; the concentration of zygomycetes 3.2 CFU/m<sup>3</sup>, accounting for 0.15%; the concentration of ascomycetes 2.0 CFU/m<sup>3</sup>, accounting for 0.1%; the concentration of unidentified fungi  $2.2 \times 10^2$  CFU/m<sup>3</sup>, accounting for 9.4%.

### 2.2 Concentration and constitution of predominant airborne fungi

The predominant airborne fungi in chicken houses are *Cladosporium*, *Penicillium*, *Aspergillus*, *Candida*, *Alternaria* and *Fusarium*, their concentrations and constitutions are shown in table 1

### 2.3 Distribution, composition and concentration of predominant airborne fungi

The distribution of airborne fungi in chicken house is shown in normal school. The spores of *Cladosporium*, *Penicillium* and *Aspergillus* were mainly distributed within C and D stages (3.0–6.0 $\mu$ m), while stage A and B (>6.0 $\mu$ m) as well as stage E and F (<3.0 $\mu$ m) relatively less. The predominant airborne fungal species are

*Cladosporium cladosporioides*, *C. macrocarpum*, *C. herbarum*, and *P. chrysogenum* (table 1).

### 2.4 Concentration and distribution of common avian pathogenic fungi

The potential harm of fungal aerosol is mainly decided by the concentration and the distribution of the pathogenic fungi. More than 10 species of common pathogenic fungi were isolated in



chicken house air, including *Aspergillus* species which can cause avian aspergillosis. The finding of the high concentration of such highly pathogenic fungi as *A. flavus*, *A. fumigatus* and *A. niger* is of epidemic importance. On the other hand, it was found that the isolation rate of mycotoxin-producing isolates like *Fusarium sporotrichioides*, *F. graminearum* and the *F. moniliforme*, is very high, their secondary metabolites may cause trichothecene toxonosis in poultry, *Candida albicans* is the pathogen of thrush, *Histoplasma capsulatum* and *Cryptococcus neoformans* may cause infection of depth tissues of human beings and poultry, *Microsporium gallinae* is the pathogen of favus, but *Penicillium islandicum* and *Aspergillus ochraceus* may cause avian toxicosis.

The predominant populations of these pathogenic fungi are distributed on different stages in the air sampler, the species on stage A are primarily represented by *F. graminearum* and *A. flavus*, *A. niger* and *C. albicans* are inferior; the species on stage B primarily by *A. niger* and *A. flavus*, the inferior is *F. sporotrichioides* and *P. islandicum*; the species on stage C primarily by *A. fumigatus*, the inferior *A. flavus*, *A. niger* and *F. moniliforme*; the species on stage D primarily by *A. fumigatus*, the inferior *P. islandicum*, *A. niger* and *A. flavus*; the species on stage E primarily by *F. sporotrichioides*, inferior *Microsporium gallinae*; the species on stage F primarily by *A. flavus*, inferior *C. albicans* (table 2).

*A. ochraceus* which appears less frequent, is mainly distributed on stage A, its distribution on stage B is inferior. *Cryptococcus neoformans* is mainly distributed on stage C, its distribution on stage A and B inferior; *Histoplasma capsulatum* is mainly distributed on stage D, its distribution on stage E inferior (table 2).

### 2.5 The airborne fungi with lower frequencies

Besides the above airborne fungi, the following fungi were infrequently isolated in the chicken houses, *Absida corymbifera*, *A.melleus*, *A. spinosus*, *Coccidioides immitis*, *Eurotium herbariorum*, *Exophiala spinifera*, *Fusarium larvarum*, *F. nivale*, *F. oxysporum*, *Mucor angulisporus*, *Penicillium cyclopium*, *P. paxilli*, *Sporothrix schenckii*, *Torulopsis glabrata*, their average concentration is 2.0 CFU/m<sup>3</sup>.

**Table 1.** Concentration and constitution of dominant airborne fungal species in chicken house

No.	Fungal species	Concentration CFU/m <sup>3</sup>	No.	Fungal species	Concentration CFU/m <sup>3</sup>
1	<i>Cladosporium cladosporioides</i>	347.5	18	<i>C. tropicalis</i>	23.6
2	<i>C. macrocarpum</i>	164.9	19	<i>Trichosporon beigeli</i>	23.6
3	<i>C. herbarum</i>	141.3	20	<i>C.albicans</i>	21.6
4	<i>Penicillium chrysogenum</i>	109.9	21	<i>F. graminearum</i>	21.6
5	<i>Candida pseudotropicalis</i>	108.0	22	<i>P. islandicum</i>	17.7
6	<i>Aspegillus flavus</i>	106.0	23	<i>Rhodotorula mucilaginosa</i>	17.7
7	<i>A. fumigatus</i>	92.3	24	<i>P. roqueforti</i>	15.7
8	<i>A. niger</i>	86.4	25	<i>F. moniliforme</i>	13.7
9	<i>P. rubrum</i>	58.9	26	<i>Monilia sitophila</i>	13.7
10	<i>P. tardum</i>	56.9	27	<i>P. citrinum</i>	13.7
11	<i>P. implicatum</i>	39.3	28	<i>P.oxalicum</i>	13.7
12	<i>Paecilomyces varioti</i>	37.3	29	<i>Scopulariopsis brevicaulis</i>	13.7
13	<i>P. citrea-viride</i>	35.3	30	<i>Trichoderma viride</i>	13.7

**Table 1.** Continuation

No.	Fungal species	Concentration CFU/m <sup>3</sup>	No.	Fungal species	Concentration CFU/m <sup>3</sup>
14	<i>P. multicolor</i>	31.4	31	<i>P. rugulosum</i>	11.8
15	<i>A. versicolor</i>	29.4	32	<i>Cryptococcus neoformans</i>	9.8
16	<i>P. urticae</i>	29.4	33	<i>Curvularia lunata</i>	7.9
17	<i>Fusarium sporotrichioides</i>	25.5		Total	1752.9

### 3. DISCUSSION

#### 3.1 The effect of sampling methods on concentration of airborne fungi

The air samples used in this study were made by means of 6-stage Andersen sampler, and it is concentrated on the active airborne fungal spores which are easily inhaled by poultry or human beings. However the traditional medium-exposing method is applicable to the sedimentation particles of microorganisms in the air. Therefore, it is incomparable between the concentration of airborne fungi in the article and that acquired by medium-exposing method.

The concentrations of airborne fungi in different conditions are different although the same air sampler was taken. The present study indicates that the concentration of airborne fungi in chicken houses is higher than in human living room (1167CFU/m<sup>3</sup>; Hu Xiao-xuan et al., 1999) by 1147 CFU/m<sup>3</sup>, and higher than in the space outside by 867 CFU/m<sup>3</sup>. The present results are similar to those acquired by means of CD-I air sampler and JWL-I micro-organism sampler in duck farm ( $2.1 \times 10^3$ CFU/m<sup>3</sup>; Ho Zhi-hui, 1994). All these results showed that the poultry raising farms have imposed a threat on surrounding environment.

#### 3.2 The biodiversity of airborne fungi in chicken house

The airborne fungi in chicken house are mainly composed by mitosporic species; it is identical with the rule of fungal distribution in wild nature. It is indicated that there is no special vent between the chicken house and the surrounding environment. Because the doors of the chicken houses are frequently opened, there is no air-filtration instalment; it therefore leads to the evident enhancement in chicken house of the fungi which are typical in outdoors environment, such as *Cladosporium* spp. The lower concentration of such common zygomycetes as *Mucor* and *Rhizopus* in chicken houses is due to the limit of the sampling method and RBC which may suppress fungal growth.

The fungal population inspected in the present study is different from those tested in the atmospheric environment by means of medium-exposing method (Huang Jiang-ju et al., 2002) and tested in the hospital environment by means of LWC-1 air sampler (Huo Yun-yan et al., 1994), and it is also different from the results obtained in atmospheric environment by means of 2-stage Andersen air sampler (Chen Mei-ling et al., 2000) but the difference is not evident. Because different sampling methods are designed for different objectives, the obtained fungal populations are naturally different. Moreover, the species of airborne fungi in different environments are apt to the influence of the sampling microclimate. The fungal species are affected by their origin, animal-raising concentration and the structure of raising houses. The distribution of airborne fungi in chicken houses is of interest to microorganism researchers.

*Aspergillus flavus*, *A. fumigatus*, *A. niger* and other conditional pathogenic fungi are commonly isolated in the chicken houses; however the most virulent airborne fungus *A. terreus* which was reported to cause avian aspergillosis failed to be isolated in the present study. It may be due to the less fortune or the small size of samples limited by factitious factors.

### 3.3 The distribution characteristic of airborne fungi in chicken houses

The distribution of airborne fungi in the chicken houses shows normal logarithm, it is basically consistent with the distribution rule of airborne fungi in Nanjing (Chen Ming-xia, 2001) and Beijing (Fang Zhi-guo, 2004). Especially the pathogenic fungi, such as *Aspergillus fumigatus* distributed on stage C with a peak, while *A. niger* and *A. flavus* mostly on stage B. The fungi float in the air mainly in the form of single spores, the spore size is the key factor to discriminate all levels of aerosols. In the current study *A. flavus* is the predominant group of fungi on stage F, it is an accidental result (only once), and it is possibly due to artificial factor.

### 3.3 The latent harm and warning of pathogenic fungi in chicken houses

The current research work is different from other studies; it is focused on the concentration and diversity of the airborne fungal spores which can be inhaled by animals and human beings. It is known that the fungal spores larger than 8.2  $\mu\text{m}$  in diameter are usually detained outside the nose cavity, and the larger spores may fall down by gravity, only the active fungal spores which can be inhaled into the depth tract of respiratory system impose a threat to the peoples' and animals' health.

The fungal spores collected by means of 6-stage Andersen Air Sampler within stage A–B ( $> 6 \mu\text{m}$ ) may get down to the small bronchus, the spores within stage C–E (1–5  $\mu\text{m}$ ) may invade the pulmonary alveolus directly, the spores at stage F are extremely thin granule ( $< 0.65 \mu\text{m}$ ). If the fungal spores are less than 0.4  $\mu\text{m}$  in diameter, they are easily expired with current. The concentration of active fungal spores within stage C and E is of biological importance. The results of the current study indicate that *A. fumigatus* is a latent threat to chickens in the investigated houses because its concentration peak is just on stage C and it is much higher than *A. flavus* and *A. niger*.

Although the concentration of *Aspergillus fumigatus* is very high in the investigated chicken houses, aspergillosis did not happen within the chickens. It may be due to the following reasons: firstly, the concentration of *A. fumigatus* is much lower and has not reached to the concentration that can cause aspergillosis with 3 magnitudes less, but the long-term contact of chickens with lower dosage of the pathogen inevitably results in slower sub-clinic symptoms, such as losses of appetite, slow-growing, low quantity egg production, immunity failure and so on, these aspects remain to be further studied. Secondly, the investigated chickens are grown-up ones (to be eliminated); they may have acquired certain immunity to the pathogenic fungus. These results provide a warning to the owner that the air disinfection in chicken houses should be tightened; otherwise it may affect the young chickens' survival. It has been found that the happening of avian aspergillosis is associated with the structure of chicken houses. Richard et al. (1984) had proven that the fungal concentration in the enclosed chicken houses could be reduced by opening the window to ventilate in spring. Reece et al. (1986) reported that the fungal diseases in chicken houses could be decreased by 75% if the methods such as reducing dust and forcing ventilation in the chicken houses were taken. Therefore, these results could provide as the scientific basis for how to control the air quality of enclosed chicken houses or those without ventilation facilities.

It is well known that *Candida albicans*, *Histoplasma capsulatum*, *Cryptococcus neoformans* and *Coccidioides immitis* can cause infection of the depth tissue which human beings and poultry can suffer from. Although their inspection rate is very low, they have a particular significance in public sanitary. They should be paid with enough attention because they impose a latent harm to the health of the related persons engaged in poultry industry.

## 4 EXPECTATIONS

### 4.1 Strengthening the formulation of pollution criterion of airborne fungi

By far there has been no pollution criterion of airborne fungi in chicken houses which can be referenced to evaluate the air quality. By comparison of the fungal concentrations in wild environment ( $1.0 \times 10^3$  CFU/m<sup>3</sup>) and in chicken houses ( $1.3 \sim 3.4 \times 10^3$  CFU/m<sup>3</sup>), it is found that the concentration of airborne fungi in chicken houses is very high. It still needs further research to formulate the detailed pollution criterion for poultry raising environment

### 4.2 Strengthening the research on the harm of *Fusarium* and its mycotoxins to poultry

Richard & Debay (1995) discovered that if turkeys were infected by *Aspergillus fumigatus*, a mycotoxin called gliotoxin toxin was produce in the infection process, and the concentration of gliotoxin in partial tissues is more than  $6 \times 10^{-6}$ , they thought there was relationship between aspergillosis and gliotoxin. This viewpoint reminds us that the dominant population of *Fusarium* in chicken houses should be given enough attention. Recently there are more and more reports about avian toxicosis related to *Fusarium*. For example, one report from one American State University (Guo Ji-ying, 1994) found that the toxins produced by *Fusarium* could change the chicken's productivity and immunity. It also indicates that mycotoxins could trigger the infection of poultry to some diseases. Therefore, the finding of the high concentration of *Fusarium* in chicken houses and the harm of their toxins should be given enough attention.

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## THE AIRBORNE FUNGI FROM INDOOR AIR OF ANIMAL HOUSES

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### ABSTRACT

The airborne fungal concentrations, sizes and compositions as well as the dominant genera in animal houses were investigated. Andersen-6 stage microbe sampler and RBC as medium were used to collect fungi aerosol from indoor air. The total number of airborne fungi was determined and their species were identified by morphological characteristics. At same time the aerodynamic analysis of the airborne fungi was also conducted. Altogether  $7.77 \times 10^3$  CFU cultivable fungal particles were incubated. The concentrations of fungi aerosol in chicken, pig, rabbit and cow houses were  $2.39 \times 10^3$ ,  $2.51 \times 10^3$ ,  $1.764 \times 10^3$  and  $1.66 \times 10^3$  CFU/m<sup>3</sup> air respectively, with the mean value of CMD (count median diameter) 3.02, 3.52, 3.29 and 3.39  $\mu\text{m}$ , GSD (geometric standard deviation) 2.03, 1.71, 1.70 and 1.69 respectively. The predominant fungi in all sampling animal houses were *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium* and *Fusarium* among the 21 genera of identified fungi. It is concluded that the airborne fungal concentrations in the animal stables surroundings were much higher than those of wild fields and common rooms. The fungal particles are easy to be inhaled into deep respiratory tract. The dominant genera of aerosol fungi in animal stables are closely related with fungal infection and mycotoxicoses.

**Keywords:** animal houses; airborne fungi; dominant fungi; harm assessment

### INTRODUCTION

Fungi aerosol which is procreated continually in animal raising house is not only endangered to the feeders and domestic animals, but also cause environment pollution<sup>[1]</sup>. Some veterinarians and feeders are very easily infected respiratory diseases who were exposed in the fungal aerosol, 13% veterinarians in Argentina are reported be infected in this way<sup>[2]</sup>. Now the total number of 318 pathogenic fungi and 420 metabolized mycotoxins, which have been identified, would make human and animals grow slowly, immunosuppressant, organ function let down, even death of mycotoxins<sup>[3]</sup>.

Air fungal composition, concentration and particle sizes were the three key harm of fungal aerosol. Many reports, such as Lanzhou Veterinary Institute<sup>[3]</sup>, showed that the harm were closely correlation with fungal concentration. The airborne fungi size was very closely correlated with the infection and endangerment of airborne fungal particles as well. 50% young turkeys were dead of *Aspergillus fumigatus* and none by *A. flavus* when two groups young turkeys were infected by same dose were found by Richard<sup>[4]</sup> and other investigations. The isolation rate of *A. fumigatus*

was 4 times of *A. flavus* in the infection turkey lungs, and more serious pathological symptom in lung in extent. The fact has been testified by Gao etc<sup>[5]</sup>. that *A. fumigatus* was more harmful than *A. flavus* and *A. niger*, therefore it deeper distance in aspiratory tract. In general, the smaller size of the fungal particles was more endangered than the bigger size by same dose of inhalation<sup>[2]</sup>.

Recently there are many study reported about active fungal particle harm in some hospitals, living rooms and public areas, but through new searched results, there is no reports about fungi aerosol in raising farms as professional disease pathogens.

## 1. MATERIALS AND METHODS

### 1.1 Sampler and culture medium

International standard ANDERSEN grade-6 sampler, air ventilation 28.3 L/min<sup>[6]</sup>, and RBC(rose Bengal chloromycetin)<sup>[7]</sup> for culture medium.

### 1.2 Sample collection

Inhalation fungal amount by human and animal were expressed by fungal CFU/min, which human or animal respiration amount (m<sup>3</sup>/min) multiply fungal concentration inhalation into mini-bronchia and alveolus. Fungal particles on stage-6 ANDERSEN from A to B stage (>6μm) could be invaded in mini-bronchia, and from C to F (<5μm) in alveolus. Fungal concentration invaded in mini-bronchia or alveolus equal percentage from A to B or C to F multiply the total concentration of sample.

Samples were collected at 50cm height from ground and for 2–4 minutes in three different structure blocked houses of chickens and pigs, one of rabbit, two semi-blocked houses of rabbit, and three opened houses of cow respectively in Shandong province. According to three times sampling in every house, 3–5 samples collected each time per week, 15 samples were gained from chicken houses and 9 samples from other animal house.

### 1.3 Incubated methods

Samples were incubated for 72 h at 25°C, and taken account of CFU(colony forming unit), and corrected the account of CFU after 7 days, then that is the real number of fungal aerosol particles on every grade of sampler.

### 1.4 Factors detection of sampling environment

Thermoscope and hygrometer (made in China) were used to detect the temperature and humidity in sampling environment.

### 1.5 Result express and relation numeration

#### 1.5.1 Airborne fungal concentration expression:

Fungal clone forming unit (CFU) in the air per ster (CFU/ m<sup>3</sup>) were used to express the airborne fungal concentration as follow:

$$\text{CFU/ m}^3 = \frac{\text{Total amounts of 6 flat plates}}{28.3\text{L/m}^3 \times \text{sampling time}(\text{min})} \times 1000$$

### 1.5.2 Airborne fungi size expression:

Total CFU of 6 stages of sampler divided by amount of CFU in every grade are every grade percentage.

1.5.2.1 Airborne fungal particle size was expressed by count median diameter(CMD).

Percentages of every stage added up stage by stage from F to A were accumulation percentage of every stage. Then linearity regression equation was calculated through accumulation percentage as x-axis and effective capture diameter (ECD,  $\mu\text{m}$ ) as y-axis, Y' value is CMD when X equal 50%<sup>[1,8]</sup>.

1.5.2.2 Airborne fungal particles disperse degree were expressed by geometric standard deviation (GSD). That is to say Y' value was divided by CMD when X equal 84.13% in the linearity regress equation<sup>[1,8]</sup>.

## 2. RESULTS

### 2.1 Sampling environment condition, fungal particle size, distribution and concentration.

#### 2.1.1 Fungal concentration and environment factors:

Fungal concentration in closed chicken house were  $1.8\sim 3.0\times 10^3$  CFU/m<sup>3</sup> when temperature changed less than 3°C, raising animal density as 5.9–10.2 ones per m<sup>2</sup>, humidity as 47~73%. Fungal concentration in closed pig house were  $2.3\sim 2.7\times 10^3$  CFU/m<sup>3</sup> when raising density as 5–10 m<sup>2</sup> per one. Especially in semi-opened rabbit house, fungal concentration were  $1.1\sim 2.7\times 10^3$  CFU/m<sup>3</sup> when raising density as 0.3~2.7 ones per m<sup>2</sup> and little change in temperature and humidity. Fungal concentration in opened cow house was  $1.6\sim 1.8\times 10^3$  CFU/m<sup>3</sup> when raising density as 10~15 m<sup>2</sup> per one(table1).

#### 2.1.2 Airborne fungal particle characters:

Fungal particles distribution apex in sampling sites was at stage-D (1.0~2.0 $\mu\text{m}$ ) with 23.4~36.3% excepted pig house at stage-C. CMD in every sampling site were 2.9~4.1 $\mu\text{m}$ , and GSD as 1.7~2.3. No significant between different sampling sites( $t=0.06$ ,  $P>0.05$ )(Table1).

**Table 1.** The concentration of aerosol fungi as well as the characteristic of sampling environment and fungal particles ( $\times 10^3$  CFU/ m<sup>3</sup>)

	Close house						semi-close house			open house		
	chicken 1	chicken 2	chicken 3	pig1	pig2	pig3	rabbit1	rabbit2	rabbit3	cow1	cow2	cow3
Fungal concentration	1.8	3.0	2.4	2.3	2.5	2.7	2.7	1.5	1.1	1.6	1.8	1.6
raising density	5.9	10.2	8.3	10	8	5	0.3	3	2.7	10	15	12
temperature(□)	25.5	24	22.5	23	25	22.5	21.5	19	21	30	25	28
humidity (%)	47	73	51	52	53.5	50	65	46	48	40	45	43
CMD( $\mu\text{m}$ )	4.1	3.3	2.6	3.6	3.6	3.4	2.9	3.3	3.7	3.5	3.4	3.3
GSD	1.6	1.8	2.3	1.7	1.7	1.8	1.8	1.7	1.6	1.7	1.7	1.7

(chicken: n=15,others: n=9); raising density (chicken and rabbit: ones/m<sup>2</sup>; pig and cow: m<sup>2</sup> /one)



### 2.1.3 Concentration and fungal particles distribution:

According to the fungal distribution on different stage, concentration of 1.0–2.0 $\mu\text{m}$  fungal particles was 5.3–6.8 $\times 10^2$  CFU /m<sup>3</sup>. Concentration of less than 5 $\mu\text{m}$  fungal particles that could invade directly into alveolus was 2 times than concentration of more than 6 $\mu\text{m}$  fungal particles invaded mini-bronchia. Percentage of less than 8.2 $\mu\text{m}$  (from stage-B to F) fungal particles that could invade respiration under nose were 79.5~97.6%, and into alveolus as 58.1~73%, and into mini-bronchia as 27.0~47.9% (Table2).

**Table 2.** The concentration ( $\times 10^2$  CFU /m<sup>3</sup>) of fungi and fungal particle distribution (%) in the sampling place (chicken houses: N=15, others: N=9)

	Stages of sampler						bronchia	alveolus
	A	B	C	D	E	F	A□B	C□D□E□F
chicken house	3.3(14.8)	4.4(18.4)	5.1(21.6)	5.5(23.4)	4.0(16.1)	1.5(5.7)	7.8(33.2)	16.1(66.8)
pig house	4.6(20.5)	4.8(21.4)	5.9(25.9)	6.8(19.0)	2.5(11.1)	0.5(2.1)	9.5(41.9)	15.7(58.1)
rabbit house	2.2(12.4)	2.5(14.6)	3.8(22.0)	6.5(36.3)	2.4(13.1)	0.3(1.6)	4.7(27.0)	13.0(73.0)
cow house	2.3(18.0)	2.8(21.3)	4.7(24.6)	5.3(27.7)	1.4(7.6)	0.2(0.8)	5.1(39.3)	11.5(60.7)

### 2.2 Fungal amount invaded into different respiration tract

Human respiration energy were calculated by 6.94E-03 m<sup>3</sup> per min (10m<sup>3</sup>/24h<sup>[2]</sup>), and of chicken, pig, rabbit, and cow by 23.5, 12, 12.5 and 20 ones per min under quietude respectively, and aerate amount per minute by 8.46E-04, 2.88E-02, 6.0E-04 and 1.44E-01m<sup>3</sup> per min<sup>[14]</sup>. Living fungal particle amount that invaded into human mini-bronchia exposure sampling sites were 3.3~6.6 CFU, and into alveolus 7.9~11.1 CFU, and into deep respiration ducts 11.4~17.7 CFU per minute (Table3).

**Table 3.** The amount of aerosol fungi arrived in the different respiratory tracts of human and animals in sampling places ( CFU/ min)

	chicken house		pig house		rabbit house		cow house	
	worker	chicken	worker	pig	worker	rabbit	worker	cow
bronchia	5.4	0.7	6.6	27.4	3.3	0.3	3.5	73.0
alveolus	11.1	1.3	10.9	45.2	9.0	0.8	7.9	165.6
deep respiration tract	16.5	2.0	17.7	72.6	12.3	1.1	11.4	238.6

### 2.3 Dominant fungi in the farming environment

Fungal aerosol of 12 animal houses was detected. 7773 CFU were captured from 252 flat plates of 42 samples after isolation and purification according to genus identification standards<sup>[15, 16, 17]</sup>. Total 21 geniuses were isolated. *Aspergillus*, *Penicillium*, *Alternaria*, *Cladsporium*, *Fusarium* et al. (Table 4) Which were found as domination fungi in sampling environment, and the others were *Acremonium*, *Bipolaris*, *Acremonium*, *Botrytis*, *Coniothyrium*, *Curvularia*, *Graphium*, *Mucor*, *Rhizopus*, *Myrothecium*, *Paecilomyces*, *Phoma*, *Rhodotorula*, *Saccharomyces*, *Scopulariopsis*, *Scytalidium* and *Trichoderma*.

**Table 4.** The categories and amount or CFU ( comparison%) of the advantage fungi genera in different farming environment ( n=42)

Fungi name	The amount of fungal colonies CFU (percentage)				
	chicken house	pig house	rabbit house	cow house	total
<i>Penicillium</i> spp.	304(12.7)	384(22.3)	204(10.1)	345(20.9)	1237 (15.9)
<i>Aspergillus</i> spp.	405(17.0)	196(11.4)	547(27.1)	139(8.4)	1287 (16.6)
<i>Fusarium</i> spp.	153(6.4)	68(3.9)	134(6.7)	95(5.8)	450 (5.8)
<i>Cladsporium</i> spp.	287(12.0)	0(0)	340(16.9)	178(10.8)	805 (10.4)
<i>Alternaria</i> spp.	334(14.0)	265(15.4)	236 (11.7)	289(17.5)	1124 (14.5)
<i>Tichoderma</i> spp.	90(3.7)	47(2.7)	134(6.7)	128(7.8)	399 (5.1)
<i>Rhodotorula</i> spp.	230 (9.6)	0(0)	25(1.2)	54(3.3)	309 (4.0)
<i>Paecilomyces</i> spp.	24(1.0)	114(6.6)	104(5.2)	119(7.2)	361(4.6)
<i>Saccharomyces</i> spp.	0(0)	121(7.0)	86(4.3)	129(7.8)	336(4.3)
<i>Curvularia</i> spp.	25(1.0)	66(3.8)	0(0)	0(0)	91(1.2)
Others	533(22.3)	464(26.9)	205(10.2)	172(10.4)	1374 (17.7)
Total	2385(30.7)	1725(22.2)	2015(25.9)	1648(21.2)	7773

### 3. DISCUSSION

Aerosol fungal concentrations were influenced by many factors such as animal habit, weather, sanitation condition and illumination. As results, aerosol fungal concentration was controlled by man-made measure, such as house structure, raising density, temperature and humidity. The higher raising density, the higher fungal concentration, this is same as the report by Huang (1977) for the readers in Library. The reason why concentration is highest in rabbit house with lowest density was temperature and humidity in blockhouses in favour of fungi increasing, bad air-conditioned. It was obvious by the cooperation to find the fungal concentration in blocked chicken and pig house was higher than in semi-blocked rabbit house and opened cow house, this result is consistent with B.W Karnick 's report which death rate of avian aspergillosis could be reached 50% in a farming house and little death as outdoor breeding. Pinello<sup>[13]</sup> improved that fungi group concentration could be reduced by window opening in blocked chicken house in spring, and incidence of avian mycosis was dropped 75% by less dust and better ventilation. so fungal pollution in breeding environment could be improved by human measures.

Stage-6 ANDERSEN sampler was made according to human respiration structure and aerodynamics characteristic of airborne particles. Capture dynamics diameters of airborne particles from A to F were >8.2µm, 6.0~8.2µm, 3.0~6.0µm, 2.0~3.0µm, 1.0~2.0µm, <0.65µm in turn<sup>[9, 19]</sup>. Capture efficiency and particle distribution of air sampler in China is the same as in America. It was well known that 20–30µm particles could invade into nose and upper respiration tract (bronchia), 6–10µm particles into mini-bronchia, 1–5µm particles into deep lung (alveolus)<sup>[10, 20]</sup>. 0.3~15µm living aerosol particles could be captured by ANDERSEN sampler which were serious harm on human and animal, especially sediment rate of 5µm particles much more higher(>90%). Bigger size and higher sediment rate of particles, which settle down in the air or block out of nose, were captured by conventional sediment method. Reported concentration was different for two sampling methods because they captured different size particles.

$2.2 \times 10^6$  CFU fungi per gram were found in cultivated lung when young turkeys would die out in 5 days, and less than  $5.2 \times 10^5$  CFU fungi per gram would die in 3–4 days, and death rate low down. That is to say young turkeys would die out of inhalation of 305 CFU/min. It was obvious

that chicken could not suffering mycosis at 1.3 CFU/min of sampling chicken house, but avian mycosis could be forecasted by fungal aerosol detection. As result, fungal concentration in detecting chicken house were 2.5 times than in outdoor environment ( $1037.5 \text{ cfu/m}^3$ <sup>[11]</sup>), and 2.2 times than in living room ( $1167 \text{ cfu/m}^3$ <sup>[6]</sup>), that is to say, one of the important pollution of atmosphere was raising environment. Fungal concentration in sampling places could not cause human or animal urgent harm, but exposure in the low fungal concentration for a long time would suffer chronic mycosis, and susceptibility to other diseases was intensity, which need to study further more.

The difference of CMD value of each sample is possible related with different source of collected fungal particles, house temperature, humidity, illumination and animal activity. The reason why these samples average CMD value was 1.5–2.0 times smaller than bacterium (supplied in our Lab.) was that fungal particles was in existence in the air as single spore and the bacterium gathered together or adhered to dust in the air, so the fungal particles was easier to enter the depth of respiration ducts than bacterium because its GSD value is over 1.6 and its distribution was larger as well<sup>[1]</sup>.

High level of biodiversity was found in all three farms. The dominant species correlate closely with fugal infections. The most frequent fungal aerosol belongs to genus *Aspergillus*, some of which are opportunistic pathogens. For example, *Aspergillus fumigatus* and *A. terreus* may infect human and animals suffering aspergillosis. Animal tests have shown that some *Aspergillus* (e. g. *A. flavus*, *A. parasiticus*, *A. versicolor*) may produce aflatoxins that induce tumour or reduce white blood cells. The second most frequent species belong to genus *Penicillium* that sometimes also infects human beings who are affected by leukaemia or lymphoma. Some species may infect brain or lung, producing ochratoxins. Third frequent genus is *Alternaria*, which may cause skin infection, hypersensitivity pneumonitis, or asthma. Some species in this genus may also produce mycotoxin that induces esophageal cancer. The fourth frequent is genus *Acremonium*, which may cause chromomycosis or phaeohyphomycosis. They commonly infect brain or skin. The fifth frequent is genus *Fusarium*, which commonly contaminates food and feed. When environment is compromised, *Fusarium* may produce mycotoxin. Some species induce skin or cornea ulcers. In rare case, *Fusarium* is associated with cancer<sup>[7]</sup>. Virulence of different species varies widely. Because resistance of the body to fungal infection also plays a crucial role, it is necessary to further study the pathogenic ability of fungal aerosol and body immunity.

#### 4. CONCLUSION

It was found through this conclusion that the fungal concentration in breeding house is higher than outdoor and indoor environment. The concentration could be reduced by way of choosing opened or semi-blocked structure animal house. Adjusting the temperature and humidity could control fungal concentration. The airborne fungal spores can be easily inhaled into the deep respiration tract than bacterium. The fungal concentrations of environment were changed with function of the places, and the dominant fungus has close relation with mycosis and toxicosis.

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## ANTIBIOTIC RESISTANCE OF AIRBORNE *ESCHERICHIA COLI* FROM HEN HOUSE AND RABBITRY AND THEIR SPREADING TO SURROUNDINGS

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### ABSTRACT

The Indoor and outdoor (windward 100 m and downwind 10, 50, 100, 200, 400, 600, 800 m) air samples in one rabbitry and one hen house in Shandong province, China were collected by two 6-stage Andersen microbial samplers or two Reuter-Centrifugal samplers, and the total number of airborne aerobic bacteria and concentrations of airborne *Escherichia coli* were measured. The concentrations of indoor airborne aerobic bacteria of the rabbitry were from  $6.1 \times 10^3$  to  $2.0 \times 10^4$  CFU/m<sup>3</sup> air, and of the hen house were from  $9.7 \times 10^4$  to  $4.9 \times 10^5$  CFU/m<sup>3</sup> air. The concentrations of airborne *Escherichia coli* of the rabbitry and the hen house were from  $3.5 \times 10^2$  to  $4.9 \times 10^2$  CFU/m<sup>3</sup> air and from  $9.5 \times 10^2$  to  $1.3 \times 10^3$  CFU/m<sup>3</sup> air, respectively. The concentrations of outdoor airborne aerobic bacteria and airborne *Escherichia coli* declined rapidly from 10 m to 800 m. The indoor airborne concentrations of aerobic bacteria in both hen house and rabbitry were significantly higher than those of outdoor (downwind 10, 50, 100, 200, 400, 600, 800 m) ( $p < 0.01$ ). The total numbers of airborne aerobic microorganisms of outdoor (downwind 10, 50, 100, 200 m) samples were significantly higher ( $p < 0.01$ ) than those of windward samples. Resistance against antibiotics isolated *Escherichia coli* strains from indoor and outdoor air samples were also analyzed. Twelve antibiotics were selected: norfloxacin (NOR), cefoperazone (CFP), chloramycetin (CMP), complex sulfanilamide (SXT), gentamycin (GEN), streptomycin (STR), tetracycline (TET), rifampicin (RIF), erythromycin (ERY), penicillin (P-G), tobramycin (TOB) and furantoin (Ni). The results showed all *Escherichia coli* strains isolated from indoor and outdoor (downwind 10, 50, 100, 200 m) air samples in the rabbitry, feed and feces resisted against RIF and P-G, while they were sensitive to TOB, GEN and Ni. The strains from indoor and outdoor (downwind 10, 50, 100, 200 m) air samples in the hen house, feed and feces were sensitive to CFP, GEN and Ni, and resisted against RIF, ERY and P-G. This could be concluded that indoor microbial aerosols include antibiotic resistance bacteria could transmit to surroundings by air exchanging and cause microbiological contamination of the air as well as epidemic transmission.

**Keywords:** animal house surroundings; microbial aerosol; antibiotic resistance bacteria; spreading; microbial pollution

## INTRODUCTION

Aerosol is a system of solid or liquid particles diffused in air. Particles of microbial aerosol are microorganisms. Microorganisms resulting from animal dander, faecal matter, feeds and water can accumulated and form microbial aerosols. Modern agricultural methods have changed the way animals are raised. More and more antibiotics are used to prevent and cure animal diseases, and that results in the producing of more antibiotic resistance bacteria. Microbial aerosols produced from animal houses can be transmitted to environment via air exchanging and cause microbiological contamination, diffusion of pathogens and antibiotic resistance bacteria<sup>1</sup>. This compromises the health of inhabitants of nearby societies.

*Escherichia coli* are a common flora of worm-blooded animals, and also pathogens or selective pathogens. This study measured the concentrations of airborne *Escherichia coli* and analyzed their resistance against antibiotics in order to find the mode of the diffusion of antibiotic resistant microbial aerosols.

## MATERIALS AND METHODS

### Environment of the hen house and the rabbitry

The studied rabbitry is partially closed with many open windows. The farm has four of these rabbitries that raised breeding rabbits and broiler rabbits. The rabbitries are cleaned everyday. The hen house is built in closed style with a mechanical ventilation system to help maintain air exchange and two thirds of the ground is covered with wood slats. The farm has seven of these hen houses which feed 5000 hens each.

### Sampling and analysis

Airborne microorganism samples of the rabbitry were collected from indoor and outdoor air (down wind 10, 50, 100, 200 m and windward 100 m as a comparison sample) at two days in March 2005 and May 2005. Air samples of the hen house were collected from indoor and outdoor air (down wind 10, 50, 100, 200, 400, 800 m and windward 100 m as a comparison sample) at two days in October 2005 and November 2005. Two 6-stage Andersen samplers<sup>2</sup> and two Reuter-Centrifugal samplers were used to collect the samples.

The samplers located 80 cm above the ground and operated for 1 to 8 min. The 6-stage Andersen samplers operated at 28.3 L/min and the Reuter-Centrifugal samplers operated at 40 L/min. The samplers were preautoclaved in the laboratory and disinfected by 70% ethanol-immersed cotton balls between each sampling.

Airborne microorganisms were collected onto 20 ml of agar with 5% sheep blood in 90-mm-diameter plates. The samples were incubated at 37°C for 24 h. Then the number of grown colonies was counted. The positive hole method was applied to AMS samples for correction<sup>2</sup>.

Feed and feces sampled from different site in the stalls at random.

### Isolation and identification of airborne *Escherichia coli*

Short gram-negative bacilli on the blood-agar plates were streaked in MacConky's (MAC) plates and incubated at 37°C for 24 h. Then pink colonies on MAC were applied and identified by using the API-20 E and API-20 NE system (BioMerieux).

### Isolation and identification of *Escherichia coli* from feed and feces

0.5 g of feces or feeds sample were dissolved in 4.5 ml sterilized broth and incubated at 37°C for 24 h. Streaked in MAC plates and incubated at 37°C for 24 h. Then pink colonies were applied and identified by using the API-20 E and API-20 NE system (BioMerieux).

### Antimicrobial susceptibility testing

Twenty *E. coli* isolates from each sample site, feed and feces at a day were used to test antimicrobial susceptibility by Kirby-Bauer method (only 1 strain was isolated at windward 100 m and downwind 400, 800 m outside the hen house respectively, so not tested). Twelve antibiotics were selected: norfloxacin (NOR), cefoperazone (CFP), chloromycetin (CMP), complex sulfanilamide (SXT), gentamycin (GEN), streptomycin (STR), tetracycline (TET), rifampicin (RIF), erythromycin (ERY), penicillin (P-G), tobramycin (TOB) and furantoin (Ni). The results were estimated by standards of CLSI<sup>3</sup>.

## RESULTS

### Concentrations of airborne microorganisms of the rabbitry

The concentrations of airborne aerobic bacteria of the rabbitry were from  $6.1 \times 10^3$  to  $2.0 \times 10^4$  CFU/m<sup>3</sup> indoor, from  $2.4 \times 10^2$  to  $5.0 \times 10^2$  CFU/m<sup>3</sup> at windward 100 m and from  $2.9 \times 10^2$  to  $7.1 \times 10^3$  CFU/m<sup>3</sup> at 10 to 200 m downwind. The number of airborne *E. coli* ranged from  $3.5 \times 10^2$  to  $4.9 \times 10^2$  CFU/m<sup>3</sup> indoor, from  $2.5 \times 10$  to  $7.4 \times 10$  CFU/m<sup>3</sup> at 10 to 200 m downwind and no *E. coli* was isolated at windward 100 m.

Statistic results showed the number of indoor airborne aerobic bacteria was higher than that of downwind 10 m ( $P < 0.05$ ) and significantly higher than those of downwind 50, 100, 200 m ( $P < 0.01$ ). The concentration of indoor airborne *E. coli* was significantly higher than those of downwind 10, 50, 100, 200 m ( $P < 0.01$ ). The concentrations of airborne aerobic bacteria of indoor and downwind 10, 50, 100, 200 m were significantly higher than that of windward 100 m ( $P < 0.01$ ). The number of airborne bacteria of downwind 10 m was significantly higher than that of downwind 50 m ( $P < 0.01$ ). Analysis showed no significant difference among the airborne concentrations of microbe of downwind 50, 100, 200 m ( $P > 0.05$ ). Bioaerosol concentrations of the rabbitry and its surroundings were given in table 1.

**Table 1.** Concentrations of airborne microorganisms measured in the rabbitry (CFU/m<sup>3</sup>)

Sampling place	n	Culturable bacteria			<i>Escherichia coli</i>		
		Max.	Min.	Median	Max.	Min.	Median
Indoor	8	$2.0 \times 10^4$	$6.1 \times 10^3$	$9.5 \times 10^3$	$4.9 \times 10^2$	$3.5 \times 10^2$	$4.1 \times 10^2$
Downwind 10 m	8	$7.1 \times 10^3$	$2.6 \times 10^3$	$5.6 \times 10^3$	7.4×10	4.8×10	6.9×10
Downwind 50 m	8	$9.6 \times 10^2$	$4.7 \times 10^2$	$5.6 \times 10^2$	6.4×10	5.0×10	5.9×10
Downwind 100 m	8	$8.6 \times 10^2$	$4.7 \times 10^2$	$5.9 \times 10^2$	5.0×10	2.9×10	4.3×10
Downwind 200 m	8	$6.8 \times 10^2$	$2.9 \times 10^2$	$4.2 \times 10^2$	4.8×10	2.5×10	3.4×10
Windward 100 m	8	$5.0 \times 10^2$	$2.4 \times 10^2$	$3.5 \times 10^2$	0	0	0

n: number of samples, Max.: Maximum, Min.: Minimum, CFU: colony forming units.

### Concentrations of airborne microorganisms of the hen house

As described in table 2, the concentrations of indoor airborne aerobic bacteria and culturable *E. coli* of the hen house were from  $9.7 \times 10^4$  to  $4.9 \times 10^5$  CFU/m<sup>3</sup> and from  $9.5 \times 10^2$  to  $1.3 \times 10^3$  CFU/m<sup>3</sup>, respectively. The concentrations of outdoor airborne aerobic bacteria and airborne *E. coli* from 10 m to 800 m were ranged from  $5.8 \times 10^2$  to  $4.1 \times 10^4$  CFU/m<sup>3</sup> and from 0 to  $4.2 \times 10^2$  CFU/m<sup>3</sup>, respectively. Only 1 strain of *E. coli* was isolated from downwind 400 or 800 m air samples, and no *E. coli* was isolated from downwind 600 m. The airborne concentrations of aerobic microbe of windward 100 m were from  $8.0 \times 10^2$  to  $1.1 \times 10^3$  CFU/m<sup>3</sup>, and only 1 strain of *E. coli* was isolated.

Statistic results showed the number of indoor airborne aerobic bacteria was significantly higher than that of downwind 10, 50, 100, 200, 400, 600, 800 m ( $P < 0.01$ ). The concentration of indoor airborne *E. coli* was significantly higher than that of downwind 10, 50, 100, 200 m ( $P < 0.01$ ). The concentrations of airborne aerobic bacteria of indoor and downwind 10, 50, 100, 200 m were significantly higher than that of windward 100 ( $P < 0.01$ ). The number of airborne bacteria of downwind 10, 50, 100, 200 m were significantly higher than those of downwind 400, 600, 800 m ( $P < 0.01$ ). Analysis showed no significant difference among the airborne concentrations of bacteria of windward 100 m and downwind 400, 600, 800 m ( $P > 0.05$ ).

**Table 2.** Concentrations of airborne microorganisms measured in the hen houses (CFU/m<sup>3</sup>)

Sampling place	n	Culturable bacteria			<i>Escherichia coli</i>		
		Max.	Min.	Median	Max.	Min.	Median
Indoor	15	$4.9 \times 10^5$	$9.7 \times 10^4$	$2.7 \times 10^5$	$1.3 \times 10^3$	$9.5 \times 10^2$	$1.1 \times 10^3$
Downwind 10 m	15	$4.1 \times 10^4$	$3.6 \times 10^4$	$3.8 \times 10^4$	$4.2 \times 10^2$	$3.2 \times 10^2$	$3.7 \times 10^2$
Downwind 50 m	15	$3.7 \times 10^3$	$3.0 \times 10^3$	$3.3 \times 10^3$	$2.6 \times 10^2$	$1.7 \times 10^2$	$2.1 \times 10^2$
Downwind 100 m	15	$2.8 \times 10^3$	$2.1 \times 10^3$	$2.5 \times 10^3$	$6.1 \times 10$	$4.1 \times 10$	$4.9 \times 10$
Downwind 200 m	15	$3.8 \times 10^3$	$1.8 \times 10^3$	$2.6 \times 10^3$	$4.6 \times 10$	$3.5 \times 10$	$4.1 \times 10$
Downwind 400 m	15	$1.5 \times 10^3$	$5.8 \times 10^2$	$1.0 \times 10^3$	4.2	0	0
Downwind 600 m	15	$9.1 \times 10^2$	$6.0 \times 10^2$	$8.0 \times 10^2$	0	0	0
Downwind 800 m	15	$8.1 \times 10^2$	$6.0 \times 10^2$	$7.4 \times 10^2$	4.2	0	0
Windward 100 m	15	$1.1 \times 10^3$	$8.0 \times 10^2$	$9.0 \times 10^2$	4.2	0	0

n: number of samples, Max.: Maximum, Min.: Minimum, CFU: colony forming units.

### Results of antimicrobial susceptibility testing

All strains isolated from indoor and outdoor air samples in the rabbitry, feed and feces resisted against RIF and P-G, and some strains resisted against NOR, CFP, CMP, SXT, STR, TET and ERY (table 3).



**Table 3.** Results of antimicrobial susceptibility testing of *E. coli* from the rabbitry (%<sup>#</sup>)

Sampling place	n	NOR	CFP	CMP	SXT	GEN	STR	TET	RIF	ERY	P-G	TOB	Ni
Indoor	20	15	15	15	70	0	30	70	100	80	100	0	0
Downwind 10 m	20	15	0	15	50	0	25	50	100	90	100	0	0
Downwind 50 m	20	15	0	15	35	0	30	45	100	100	100	0	0
Downwind 100 m	20	20	0	15	45	0	15	55	100	100	100	0	0
Downwind 200 m	20	10	0	0	20	0	20	50	100	100	100	0	0
Feces	20	20	20	15	75	0	50	70	100	100	100	0	0
Feed	20	10	0	10	20	0	35	50	100	100	100	0	0

#: percentage of resistant isolates to total tested isolates.

N: number of samples

The strains from indoor and outdoor air samples in the hen house, feed and feces resisted against RIF, ERY and P-G., and some strains resisted against NOR, CFP, CMP, SXT, STR and TET (table 4).

## DISCUSSION

The level of airborne bacteria and *E. coli* in this study were different from those in many published reports<sup>4,5,6,7</sup>. It is reasonable to attribute to the structure of the stalls, feeding method, animal density and cleaning practice and frequency. Bioaerosol in stalls mostly come from the animals, microorganisms resulting from animal faecal matter, dander and feed materials are easily accumulated and aerosolized<sup>8</sup>. Proper animal density, ventilation and cleaning are effective measures to reduce the concentrations of airborne microorganisms.

The concentrations of outdoor airborne aerobic bacteria and airborne *E. coli* were lower than that of indoor and declined rapidly from downwind 10 m to 800 m. This could be concluded that indoor microbial aerosols could transmit to surroundings by air exchanging and form a higher concentration of bacteria near the farm. This microbiological contamination compromised the health of inhabitants of nearby societies. Pathogenic *E. coli* could cause hominine diarrhoea, hemorrhagic colitis and urogenital system inflammation.

**Table 4** Results of antimicrobial susceptibility testing of *E. coli* from the hen houses(%<sup>#</sup>)

Sampling place	n	NOR	CFP	CMP	SXT	GEN	STR	TET	RIF	ERY	P-G	TOB	Ni
Indoor	20	55	0	0	55	0	45	80	100	100	100	0	10
Downwind 10 m	20	0	0	0	50	0	0	50	100	100	100	0	0
Downwind 50 m	20	0	0	0	40	0	0	45	100	100	100	0	0
Downwind 100 m	20	0	0	15	35	0	30	50	100	100	100	0	0
Downwind 200 m	20	0	0	0	0	0	40	50	100	100	100	0	0
Feces	20	30	20	0	70	0	40	70	100	100	100	0	0
Feed	20	20	0	0	25	0	50	50	100	100	100	0	0

#: percentage of resistant isolates to total tested isolates.

N: number of samples

As shown in table 3, the percentages that the *E. coli* strains isolated from rabbit feces resisted against NOR, SXT, CFP, STR, ERY was 20%, 75%, 50%, 50%, 100%, respectively. These were higher than those of indoor airborne *E. coli* strains: 15%, 70%, 30%, 30%, 80%. All *E. coli* strains

from indoor air samples and feces were resisted against P-G and RIF, and the percentages they resisted against TET and CMP were equal. Similar results were got from the *E. coli* strains from the hen house and hen feces. These accorded with the routine usage of medicine of the farms which were obtained on the sampling days. *E. coli* strains resisted against medicines that used frequently and were sensitive to those rarely used. This could be concluded that airborne antibiotic resistant bacteria come from animal feces.

Results of antimicrobial susceptibility testing showed *E. coli* strains isolated from the rabbitry surroundings resisted against P-G and RIF, the same as *E. coli* strains from indoor air samples. *E. coli* strains from the hen house and its surroundings resisted against RIF, ERY and P-G. All tested isolates had similar ratio of resistance against other antibiotics. This showed outdoor airborne antibiotic resistant bacteria come from the stalls by air exchanging.

The percentage that airborne *E. coli* strains isolated from indoor air samples of the rabbitry resisted against NOR was lower than that of downwind 100 m outside the rabbitry. The percentage that indoor airborne *E. coli* strains isolated from the rabbitry resisted against ERY was lower than those of downwind 10, 50, 100, 200 m. This indicated that downwind airborne antibiotic resistant bacteria had other resources besides the rabbitry, such as nearby societies and other farms. No such results had been found from the studied hen house probably because there were no farms near it and 1000 m away from the nearest village.

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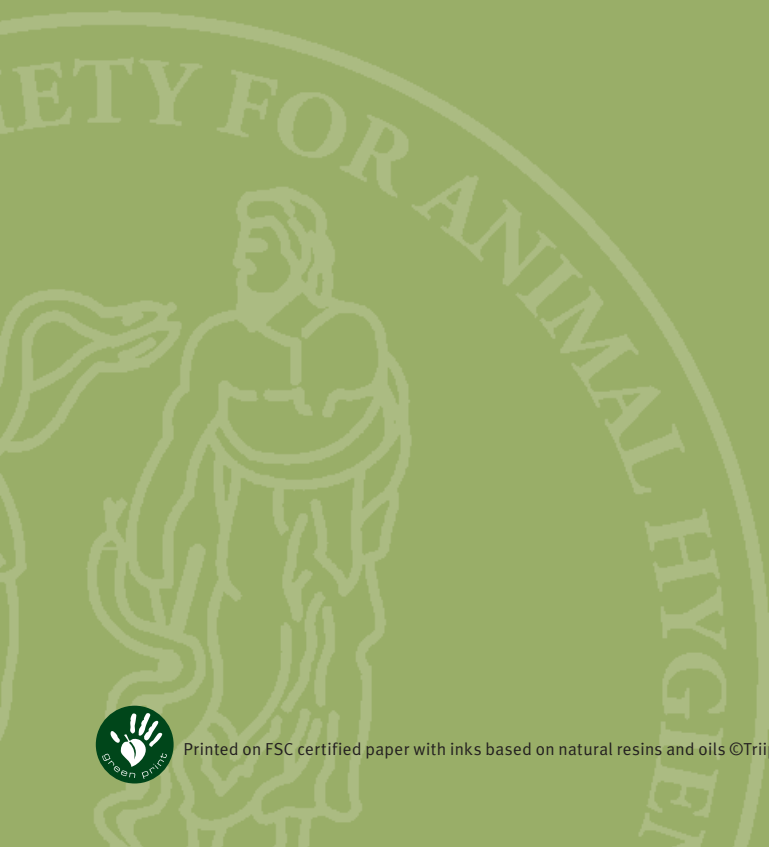


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