INDIVIDUAL BEHAVIOURAL CHARACTERISTICS IN PIGS AND THEIR CONSEQUENCES FOR PIG HUSBANDRY

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INDIVIDUAL BEHAVIOURAL CHARACTERISTICS IN PIGS AND THEIR CONSEQUENCES FOR PIG HUSBANDRY

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Proefschrift

ter verkrijging van de graad van
doctor in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
dr. C.M. Karssen,
in het openbaar te verdedigen
op dinsdag 22 februari 1994
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen

Omslag: Wim Valen

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Hessing, Manfred J.C.

Individual behavioural characteristics in pigs and their consequences for pig husbandry / Manfred J.C. Hessing.
[S.l. : s.n.]
Thesis Wageningen. - With ref. - With summary in Dutch.
ISBN 90-5485-213-5
Subject Headings: pigs; coping strategies; immunity.

Hessing, M.J.C., 1993. Individual behavioural characteristics in pigs and their consequences for pig husbandry (Individuele gedragseigenschappen van het varken en de betekenis daarvan voor de varkenshouderij).

Ph.D. thesis, Department of Animal Husbandry, Section Ethology, Agricultural University, P.O. Box 338, 6700 AH Wageningen, The Netherlands.

STELLINGEN

I

In stressvolle situaties bestaat het gemiddelde dier niet.

Dit proefschrift

II

Tepelorde van zuigende biggen is een irrelevant begrip.

Dit proefschrift

III

In stressvolle situaties hebben actieve en passive 'copers' elkaar nodig.

Dit proefschrift

IV

In de huisvesting en management van varkens in de intensieve veehouderij zijn de sociale aspecten onderschat.

Dit proefschrift

V

De consument is zich onvoldoende bewust van een sleutelrol in het welzijn van landbouwhuisdieren.

VI

De 'bottleneck' in toegepast onderzoek is de toepassing.

VII

Het feit dat er geen veeteler werkzaam is op de sectie Ethologie van de vakgroep Veehouderij van de Landbouwuniversiteit Wageningen is een riskante onderschatting van de praktijk.

VIII

Het gedrag van de veehouder is een onderschat gegeven voor de (re)produktiviteit en het welzijn van de landbouwhuisdieren.

Hemsworth, P.H., Barnett, J.L. and Coleman, G.J., 1993. The human-animal relationship in agriculture and its consequences for the animal. Animal Welfare 2:34.

IX

Het feit dat het varken in de toekomst de belangrijkste orgaandonor voor de mens lijkt te worden geeft een extra stimulans voor onderzoek naar de invloed van stress op orgaanbeschadigingen bij het varken.

X

Ter preventie van huidkanker is een gedragsverandering bij de mens noodzakelijk.

XI

Het varken voelt de intensieve houderij in de maag.

XII

Gestoord gedrag is niet onbegrijpelijk.

XIII

Het is gemakkelijker bedenkingen te uiten dan oplossingen aan te dragen.

XIV

Voor een vlotte voortgang van een proefschrift raadplege men nooit meer dan één statisticus.

M.J.C. Hessing

Individual behavioural characteristics in pigs and their consequences for pig husbandry

Wageningen, 22 februari 1994

mooi is een kooitje
met een kip erin

heel mooi ook een kooitje
met een konijn erin

met een nerts erin, met een kalkoen erin,
een kalf erin, een varken erin

maar het mooiste is eigenlijk
een kooitje met niets erin

Gebaseerd op C. Buddingh in "gedichten 1938/1970"
De bezige bij, Amsterdam, 1971.

ter nagedachtenis

aan mijn moeder

CONTENTS

		Page
GENERAL INTRODUCTION		9
CHAPTER 1	Social rank and disease susceptibility in pigs	15
CHAPTER 2	Individual behavioural characteristics in pigs	31
CHAPTER 3	Individual behavioural and physiological strategies in pigs	43
CHAPTER 4	Individual differences in cell-mediated and humoral immunity in pigs	61
CHAPTER 5	Practical implications of individual behavioural characteristics in pigs	81
GENERAL DISCUSSION		95
SUMMARY		111
SAMENVATTING		117
REFERENCES		123
LIST OF PUBLICATIONS		132
DANKWOORD		133
CURRICULUM VITAE		135

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Pigs are highly social animals whether they live in the wild or as domesticated ones under farm conditions (Craig, 1986; Gundlach, 1968; Mauget, 1981; Meese and Ewbank, 1973). In general, this social organization is based upon a dominance hierarchy that is established through agonistic interactions among group members. The eventual social position of the individual animal in a group depends on many traits like age, gender, body weight, body size, aggressiveness and social experience (Craig, 1986; McBride et al., 1965; Rowell, 1974; Rushen, 1988). But once the dominance relations have been formed agonistic interactions become less frequent, shorter and less intense, and as a rule threat behaviour alone maintains a stable social hierarchy. In a social group pigs must learn to react adequately to the behaviour of the group members and to predict the reactions of a conspecific. This "controllability and predictability" over their (social) environment reduces social tension in the group (Wiepkema and Schouten, 1988). Pigs living under intensive husbandry conditions it is far more difficult to control and predict their social environment than for the pigs living in the wild or in extensive conditions. In these latter conditions social stability of a group is most striking. In intensive husbandry the social environment of the pigs is influenced by early weaning, group size, density, group membership disruption, and a homogeneous (by age and by weight), and highly fixed and artificial group composition (Stricklin and Mench, 1987; Tennessen, 1989). Such conditions make it extremely difficult for pigs to establish and maintain a stable social order; this may result in great social friction in the group. These negative consequences, often referred to as social stress, are among the most adverse stressors that elicit physiological (Fraser, 1984), endocrinological (Mason, 1968; Mormède et al., 1988), immunological (Beden and Brain, 1985; Fleshner et al., 1989; Gust et al., 1991) and behavioural changes (Kaplan et al., 1983; Meese and Ewbank, 1972; Von Holst, 1986). These changes differ between animals of different social status (Koolhaas and Bohus, 1989; Sapolsky, 1990; Von Holst, 1986). Besides that, the social behaviour (e.g., social competition) of an animal that lives in a social organization is also determined by its individual way of handling conflict situations (i.e., coping).

Coping is the individual respons to a stressor by which harmful physiological effects of this stressor are reduced (Levine and Wiener, 1989). In view of the classical stress literature coping can be attained by: 1) removing the stressor or fleeing from it (i.e., active coping); or 2) adjusting to the stressor and accept it as it is (i.e., passive coping). Active coping attempts resemble the fight-flight response as described by Cannon (1929), which is characterized by the release of peripheral catecholamines, indicating a high sympathetic nervous adrenal-medullary activity that prepares the organism to react to a threatening situation. In contrast, the passive

coping attempts refer to the conservation-withdrawal response as described by Engel and Schmale (1972), and corresponds to a Selyean stress response, and is accompanied by an increase in adrenocortical activity (Selye, 1950). The intriguing point is that, although both mechanisms are present, each individual appears to be predisposed to one or the other coping style, presumably determined by genetic constitution and early life experiences (Suomi, 1987; Susman et al., 1989; Van Oortmerssen et al., 1985). Recent data substantiate the existence of these two different coping styles, active versus passive, in several species (Corson and Corson, 1976; Slater, 1981; Von Holst, 1986). Hence, it is likely that such idiosyncratic response pattern to a challenge is also present in pigs.

If so, these individual behavioural characteristics may be an important aspect in understanding the complex social relations among animals living in social groups. Because the natural distribution of different coping styles or types of animals in a stable social structure may not be arbitrary, this could be a guideline in how to compose a group of animals. Yet little research has been conducted to reveal possible natural patterns of group composition in pigs and how the underlying mechanisms could be applied in intensive pig husbandry.

Aim and outline of the thesis

In view of the above the aims of the present study are to investigate the following questions:

- does the social position of an individual pig in a stable social organization relate to its health status,
- do individual behavioural characteristics in pigs exist, and are these differences consistent over situations (social and non-social), and over time,
- do these individual differences relate to different external (behavioural) and internal (physiological, endocrinological and immunological) programs in pigs when stressed,
- and, may group composition, based on individual coping characteristics, influence the growing up of pigs in the intensive husbandry.

At first, individual variation in disease susceptibility and immunity of pigs is described and discussed in chapter 1 with emphasis on individual social status in the group. Because such

variations may reflect a more general individual way of coping with stressful situations, the existence of consistent individual behavioural characteristics in pigs was studied and described in chapter 2. Since vertebrates show an integrative and adaptive reactivity to changes in their external and internal milieu, the individual behavioural characteristics in pigs have been related to different behavioural, physiological and endocrine responses under stress conditions in chapter 3. Subsequently, chapter 4 describes whether the supposed individual coping styles in pigs are reflected in different cell-mediated and humoral immunity to specific and non-specific antigens when stressed. Finally, at a commercial closed farm an experiment was performed to study whether the supposed individual coping strategies could be applied in intensive pig husbandry to benefit the growing up of fattening pigs (chapter 5). In the general discussion the major findings of chapter 1 - chapter 5 are discussed with emphasis on the autonomic nervous system, the immune system, and the practical implications of behavioural studies in pigs.

Chapter 1

SOCIAL RANK AND DISEASE SUSCEPTIBILITY IN PIGS

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Accepted for publication in: Veterinary Immunology and Immunopathology (in press)

SOCIAL RANK AND DISEASE SUSCEPTIBILITY IN PIGS

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ABSTRACT

Two experiments were carried out to investigate the inter-individual variation in immune reactivity and disease susceptibility of group-housed pigs of different social status. The social status of the individual pig was determined by the outcome of social ranking fights and food competition tests. On day 75 after the start of both experiments all pigs were challenged with 0.5 ml of an Aujeszky-virus (ADV) into each nostril. Data combined from both experiments showed that mortality and morbidity after the ADV-challenge was highest among subordinates. In both experiments, a lymphocyte stimulation test, using purified Aujeszky-virus as an antigenic stimulus, showed that dominant pigs had significantly higher counts/minute than subdominant and subordinate pigs. Kendall's partial correlations showed that morbidity had been associated with high values in haematological and clinicochemical blood parameters and not with social status of the individual pig. In each experiment, maternal derived antibodies against the Aujeszky virus and the antibody level after the ADV-challenge hardly differed between pigs of different social status. MHC-typing of class I and II by iso-electro focusing of all pigs in experiment 2 showed that not all haplotypes were equally distributed among dominant, subdominant and subordinate pigs. The present study shows that there are large individual differences in immune reactivity and disease susceptibility, that appear to be related to the social status of the individual pig in a stable social structure.

Keywords: Pigs; Social status; Disease susceptibility; Immune reactivity

INTRODUCTION

Social structure in pigs is monitored by social dominance, as described in the wild boar (Gundlach, 1968) and in domesticated pigs (Meese and Ewbank, 1973; Jensen, 1982). Social dominance is established through ranking fights. The benefit of a stable social structure is that it reduces severe fighting among the individuals in the group (Kondo and Hurnik, 1990; Meese and Ewbank, 1972). It also minimizes social stress because interactions between the animals are predictable and controllable and by this reduce uncertainty (Ely and Henry, 1978; Levine et al., 1989; Mormède et al., 1988; Wiepkema and Schouten, 1988). Stress stemming from tensions in a group, like during the formation of new groups, the introduction of a new animal or when the dominant animal loses its position, strongly influences neuroendocrine processes in an individual (Dantzer and Mormède, 1985; Ely and Henry, 1978; Hansen et al., 1980; Von Holst, 1986).

Therefore, the social status of an animal is an important factor determining its neuroendocrine response to social stress (Fleshner et al., 1989; Hansen et al., 1980; Henry and Stephens-Larson, 1985). Social stress can also alter cell-mediated (Gust et al., 1991; Koolhaas and Bohus, 1989; Raab et al., 1986) and humoral immune reactivity (Beden and Brain, 1985; Fleshner et al., 1989). Large individual differences occur in the reaction to certain stressors (Benus et al., 1987; Bohus et al., 1987; Wiepkema and Schouten, 1988) and, therefore, the coping style of the individual to stressors could well be different between animals of different social status. The present study reports about individual variation in immune reactivity and disease susceptibility of pigs in relation with their social status in a stable social structure.

MATERIAL AND METHODS

Housing conditions

Two experiments were carried out in a climate controlled pig house with two fully separate rooms (Tielen, 1986). Each room was completely air-conditioned and consisted of five pens (7 m^2/pen), with a heated concrete lying area and metal slats. In both experiments artificial lights were on from 08.00 until 18.00.

Subjects

Two weeks before farrowing in each experiment ten sows (Yorkshire x Dutch landrace) were transported to the pig house and randomly divided over the two rooms. The sows farrowed within seven days. Piglets were individually weighed soon after birth, 24 hours later and further on subsequently at weekly intervals. In each experiment, two days post partum the piglets of each sow were matched pairwise by sex and weight, and randomly distributed between the two rooms. Thus weight, sex and genetic background differences between the animals of both rooms were minimized. The piglets had free access to water from two nipple drinkers and *ad libitum* food from one space feeder. A single commercial feed (energy content: 13.9 MJ ME/kg feed) was given throughout both experiments from day 10 on. Male pigs were castrated at approximately ten days of age. Each pig could be recognized by a tattoo number in the ear, a plastic ear tag and a number painted on the back. On day 35 of the experiments all pigs (experiment 1: 79 pigs; experiment 2: 90 pigs) were weaned by removing the sows. In each experiment pigs were not mixed, but remained in the same pen from birth until slaughter.

Teat order

The teat order was observed at least ten times for every litter throughout the suckling period. Teat pairs were numbered from 1 to 8, anterior to posterior.

Social status

In experiment 1, two litters (one litter/room; 10 piglets/litter) were chosen from which two pigs died during the suckling period. The social status of the remaining 18 pigs was determined by monitoring social ranking fights (approximately 350 social interactions) ad random during daily activities. Social status of each individual was determined according to the social-rank index, as described by Lee, Craig and Dayton (1982). In experiment 1 the social status of each individual pig was also determined by the outcome of food competition tests on day 50, day 65 and day 100. During 24 hours before each food competition test pigs had no access to food. A small trough was fixed to the slatted floor and contained a little amount of food. The pigs were tested pairwise in their own pen. It took about 3 hours to test all pairwise combinations (n=73; day 50 and day 65, n=43; day 100). Each confrontation was ended immediately after a clear winner and loser was evident. The other pigs of the same group remained in the same pen but were unable to interfere with the test situation. Since high positive correlations were found between social status as determined by the outcome of social ranking fights and of food competition tests, in experiment 2 social status of all pigs (n=90) was determined only according to the outcome of the social ranking fights (approximately 3200 social interactions). In both experiments pigs in each litter were ranked in three groups (high, middle and low). Due to different littersizes (8-11 piglets/sow) total number of high (dominant), middle (subdominant) and low (subordinate) pigs differed somewhat.

Aujeszky Disease Virus-challenge (ADV)

In both experiments pigs were challenged intranasally on day 75 with an Aujeszky-virus (also known as pseudorabies, strain 75V19). This strain was derived from a field isolate. The challenge was performed by infusing 0.5 ml of the virus (dose: $10^{6.2}$ Tissue Culture Infective Dose $(TCID)_{50}$ in experiment 1 and $10^{5.6}$ $TCID_{50}$ in experiment 2, in porcine kidney cell cultures) into each nostril of the pig. All sows had been vaccinated against Aujeszky-disease. The mortality after the ADV-challenge was monitored for every individual pig. Tonsils and brain of the pigs that died after the challenge were checked for ADV. Moreover, these pigs and all pigs slaughtered at the end of the experiments (experiment 1: day 105; experiment 2: day 108) were examined for pathological alterations in the lungs, heart, liver, nose, and stomach.

Only in experiment 2 morbidity after the ADV-challenge was monitored daily for each individual pig by two observers who were unaware of the pigs' individual social position. Morbidity was classified as light or severe based on clinical signs.

Humoral immunity

In experiment 1 a serum neutralisation (SN) test was performed of all pigs on day 35 (weaning), before the ADV-challenge (day 75) and four weeks after the ADV-challenge (day 103), according to standard procedures of the laboratory of the Animal Health Service in South Netherlands (after de Leeuw et al., 1982). Serum neutralisation Aujeszky-antibody titre was expressed as the $\log_2$ titre of the highest dilution of serum that completely inhibited a cytopathic effect, as determined in a microneutralisation test. The ten sows used for experiment 2 had been vaccinated with a gI-negative vaccine. With such a vaccine it is possible to distinguish between vaccinated and infected pigs (Van Oirschot and De Waal, 1987). In experiment 2 ADV-glycoprotein-I (gI)-titres (in a $\log_3$ titre) of all pigs were determined on day 35, before the ADV-challenge (day 70) and after the ADV-challenge on day 84 and day 102 as described elsewhere (Van Oirschot and De Waal, 1987).

Lymphocyte stimulation test

In both experiments an ADV-specific lymphocyte stimulation test (LST) was performed four weeks after the ADV-challenge (experiment 1: day 103; n=12; experiment 2: day 102; n=32; animals were randomly selected). Heparinized blood samples were diluted 1:1 with RPMI 1640 culture medium and layered onto 10.0 ml of Ficoll-paque (Pharmacia no 17-0840-02). Samples were centrifugated at 400 x g for 30 minutes at room temperature. At the interface of plasma and Ficoll-paque, lymphocytes were collected and washed twice with 0.15 M PBS (pH 7.2). Cells were sedimented by centrifugation at 150 x g for 10 minutes and the pellet was washed twice with RPMI 1640 culture medium. After the final wash, lymphocyte concentrations were determined and adjusted to 5 x 10^6 viable cells/ml by adding supplemented RPMI 1640 culture medium containing heat-inactivated fetal calf serum (10%). Viability of cells, which was always > 95%, was determined by trypan blue dye exclusion. The LST consisted of 100 μl of lymphocytes at 5 x 10^6 cells/ml added in quadruplicate to wells of a 96-well flat-bottom microtitre plate. Control cultures were supplemented with RPMI 1640 culture medium containing no antigen. Concentrations of 10 μg, 5 μg or 1 μg/ml of purified ADV-virus protein (75V19) was used as antigenic stimulus. All cultures were incubated at 37°C in a humidified 5% CO_2 - 95% air atmosphere for 48 hours. Cultures were labeled with ^{3}H-thymidine (0.4 μCi in 20 μl RPMI

culture medium) for an additional 18 hours. Cells were harvested for liquid scintillation counting onto filter paper with deionized water by an automated cell harvester. Lymphocyte proliferation of individual animals was calculated as the number of counts/minute (cpm) of each of the specific ADV-virus concentration minus the non-stimulated control culture.

Haematological blood parameters

In each experiment, blood samples were taken from all animals before (experiment 1: day 75; experiment 2: day 70) and four weeks (experiment 1: day 103; experiment 2: day 102) after the ADV-challenge. The number of leucocytes was counted by a Coulter Counter and expressed in 10^6/ml blood. For leucocyte-differentiation, blood smears were stained with May-Grunwald-Giemsa (in duplicate). And 100 leucocytes were microscopically counted and differentiated into lymphocytes, neutrophils, monocytes, eosinophils, and basophils, and they were also expressed in 10^6/ml blood.

Clinicochemical blood parameters

In each experiment, measurement of total protein, albumin, α-, ß-, and γ-globulin serum content of all animals was also done, as described above, before and four weeks after the ADV-challenge. Total protein content was determined by the biuret method and albumin and globulin contents were determined by electrophoresis on cellulose acetate and quantified densitometrically (Elbers et al., 1991).

MHC-typing

In a first assessment all pigs (n=90) of the second experiment were typed for swine lymphocyte antigens (SLA) class I and class II by iso-electro focusing (IEF) technique as described elsewhere (Joosten et al., 1988; Joosten et al., 1989). Porcine class I and class II precipitating antibodies' TH16 and PT85 were used (Lunney et al., 1988). SLA-class I focusing patterns were expressed as HF..; H in analogy with the porcine serology nomenclature of the class I region (Renard et al., 1988) and F for focusing, whereas SLA-class II focusing patterns were expressed as DF.. ; D in analogy with the porcine serology nomenclature of the class II region (Renard et al., 1988) and F for focusing.

Statistical analysis

All variables were checked for normal distribution using the Examine procedure within the SPSS-PC+ version 3.1 (Norusis, 1989). A multiple range test (Tukey) was used for testing

differences between social rank group (high, middle and low) and blood parameters (Norusis, 1989). Kappa statistics are given to test the measure of agreement in the outcome of successive food competition tests (Kelsey et al., 1986). The relation between social status based on the outcome of the social rank fights and the food competition tests was analyzed with Spearman rank correlations (Siegel and Castellan Jr., 1988). Other relations between variables were determined with Pearson's correlations (Norusis, 1989). If a correlation between two variables could be due to the association between the two variables and a third variable, Kendall's partial correlations are given (Siegel and Castellan Jr., 1988). Differences in mortality and morbidity after the ADV-challenge were tested with the Cross tabulation method, which measures a Chi-square (Norusis, 1989). The same procedure was used comparing different MHC-types in the high, middle and low ranking animals. Crude Odds Ratio (OR) was calculated with the use of Statistix^r. The Odds Ratio is an estimation of the relative risk factor (Rothman, 1986).

RESULTS

Social status

Social ranking fights were mainly seen during the suckling period, and reached a peak between the second and third week of age of the piglets. After this period only a few ranking fights were observed and just some minor changes occurred in the individual social status of a pig. Social status based on the outcome of these social ranking fights and the pairwise combinations in the food competition tests in experiment 1 highly correlated (day 50; $r_s(18)=0.91$, $P < 0.01$: day 65; $r_s(18)=0.89$, $P < 0.01$ and day 100; $r_s(14)=0.85$, $P < 0.01$). Moreover, kappa values indicated a high agreement between the outcome of successive food competition tests (day 50 - day 65: 95% agreement, $K = 0.89$, $P < 0.01$: day 65 - day 100; 87% agreement, $K = 0.81$, $P < 0.01$). This indicates a relatively stable social structure in each pen. Based on their social status, pigs were divided into high, middle and low social rank groups. Due to different litter-sizes, total numbers of pigs in the three rank groups in experiment 1 (two litters) were subsequently 5 (3♂/2♀), 7 (3♂/4♀) and 6 (3♂/3♀), and in experiment 2 (ten litters); 27 (14♂/13♀), 32 (13♂/19♀) and 31 (15♂/16♀); obviously gender did not contribute to the social status of subadult pigs.

Birth weight and social status. Data combined of both experiments (12 sows/108 piglets) showed a significant positive relation between birth weight and social status of the individual pig ($r_p(108)= + 0.47$, $P < 0.01$). Birth weight explained 22% of the variation in individual social status of the pig ($r^2=0.221$, $P < 0.05$).

Teat order

Teat order stabilized within the first week. Data combined of both experiments showed no significant relation between teat order and birth weight of a piglet ($r_p(108) = + 0.12$), and the same was found for teat order (anterior to posterior) and social status ($r_p(108) = - 0.09$).

Aujeszky Disease Virus-challenge

The Aujeszky virus strain (75V19) derived from the field isolate was of a much higher virulence than expected and some pigs died or showed severe clinical signs. Mortality after the ADV-challenge in experiment 1 was 13.9% (11/79) and 8.9% (8/90) in experiment 2. The Aujeszky virus could be isolated from the tonsils and the brain of these pigs. Combined data of the two experiments showed that mortality was higher among subordinate pigs (8/37 = 21.6%) than among dominant (2/32 = 6.3%) or among subdominant ones (2/39 = 5.1%) ($\chi^2 = 6.32$, $P < 0.05$). Pigs that died after the challenge and the ones slaughtered at the end of both experiments revealed no quantitative or qualitative significant differences in pathological alterations in the lungs, liver, heart, nose, and stomach between animals of different social status. In both experiments clinical signs were observed two days after the ADV-challenge. These signs consisted of an increase in sneezing-frequency, purulent nasal discharge, fever, anorexia, constipation, salivation, vomiturition and uncoordinated movements. While morbidity in experiment 1 was not classified quantitatively or qualitatively, this was done in experiment 2. In this latter experiment total number of pigs with clinical signs was higher in the low ranking group (19/27) than in the middle- (16/30) and in the high ranking group (8/25) ($\chi^2 = 6.03$, $P < 0.05$) (Table 1.1). Morbidity was also more severe in the low ranking group (17/27) ($\chi^2 = 12.00$, $P < 0.01$) than in the middle- (9/30) and in the high ranking group (5/25) (Table 1.1). As expected, odds ratios for morbidity after the ADV-challenge were highest ($P < 0.05$) for low ranking pigs (Table 1.1).

Table 1.1. The relation between social rank (high, middle or low) and morbidity (light and severe clinical signs) in number and % of the animals after the Aujeszky-challenge in experiment 2. Odds Ratios (OR) for light + severe and severe clinical signs are given.

Social rank	Number of animals	Number of animals (%) with clinical signs[a]		OR	OR
		Light	Severe[b]	light + Severe	Severe
High	25	3 (=12%)	5 (=20%)	1	1
Middle	30	7 (=23.3%)	9 (=30%)	2.43	1.86
Low	27	2 (=7.4%)	17 (=63%)	5.05	6.80

[a] Low ranking animals with clinical signs (light + severe) > middle and high ranking animals ($P < 0.05$).
[b] Low ranking animals with severe clinical signs > middle- and high ranking animals ($P < 0.01$)

Humoral immunity

Serological blood parameters.

Table 1.2. The mean (± SD) SN-titre (log_2) on day (D) 35, D75 and D103 in experiment 1 and gI Aujeszky-antibody titre (log_3) on D35, D70, D84 and D102 in experiment 2 of high, middle and low ranking pigs.

Social rank	High	Middle	Low
SN-titre D35	4.15 ± 0.43 (n=5)	4.23 ± 0.56 (n=7)	3.94 ± 0.47 (n=6)
SN-titre D75	2.66 ± 0.73 (n=5)	2.55 ± 0.36 (n=7)	2.08 ± 1.01 (n=6)
SN-titre D103[a]	6.43 ± 0.83 (n=4)	6.40 ± 1.14 (n=5)	7.50 ± 0.58 (n=5)
gI-titre D35[b]	2.22 ± 1.05 (n=27)	2.17 ± 1.14 (n=30)	2.00 ± 1.02 (n=31)
gI-titre D70[b]	1.13 ± 0.85 (n=27)	0.76 ± 0.87 (n=30)	0.65 ± 0.88 (n=31)
gI-titre D84	3.18 ± 1.02 (n=27)	3.11 ± 0.80 (n=30)	3.12 ± 0.67 (n=27)
gI-titre D102	3.86 ± 0.45 (n=25)	3.93 ± 0.47 (n=30)	3.96 ± 0.53 (n=27)

[a] SN-titre D103 of pigs in the low ranking group > pigs of the middle- and high ranking group.
[b] Blood samples of two pigs of the middle rank group were missing.

Aujeszky-antibody titre did not differ significantly between high, middle and low ranking pigs before the ADV-challenge in both experiments (Table 1.2). Merely, the antibody titre four weeks (day 103) after the ADV-challenge in experiment 1 was higher for low ranking pigs compared to middle- or high ranking pigs ($P < 0.05$) (Table 1.2). The lower Aujeszky-titre on day 75 (experiment 1) and day 70 (experiment 2) reflect decreasing maternal derived antibodies (MDA). Surprisingly, a stepwise multiple regression analysis (Norusis, 1989) showed that body weight, teat order, litter and social status did not significantly contribute to the variation in ADV-specific MDA that existed between pigs.

Cell-mediated immunity

Lymphocyte stimulation test (LST). In experiment 1 dominant pigs had a significant higher ($P < 0.01$) number of counts/minute (cpm) in the ADV-specific LST in all three Aujeszky virus concentrations than subdominant and subordinate pigs (Figure 1.1).

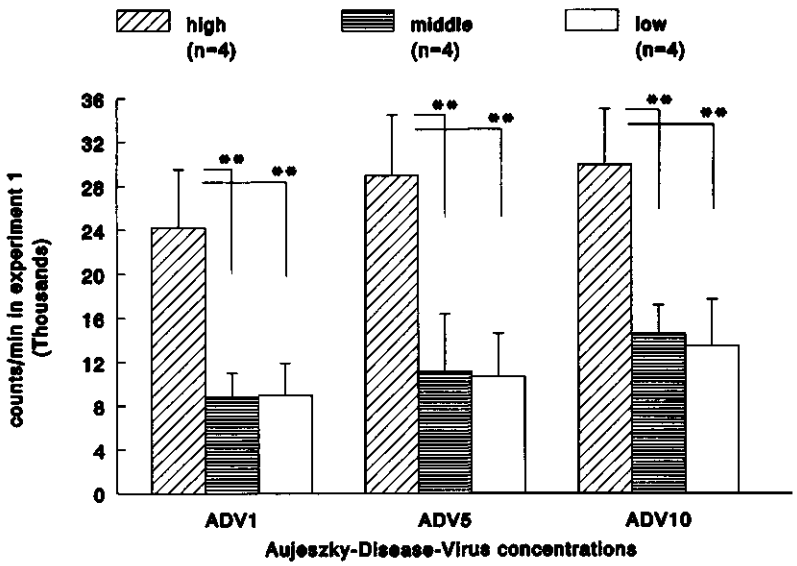

Figure 1.1. The mean number (± SEM) of counts/minute (cpm) in the ADV-specific lymphocyte stimulation test, using different Aujeszky virus concentrations (1, 5 and 10 μg/ml), minus the cpm of the control cultures (background counts) of high, middle and low ranking animals in experiment 1 (** = $P < 0.01$). Background cpm (± SEM) of high, middle and low ranking animals were, respectively, 3354 ± 938, 4089 ± 1049 and 4096 ± 369.

In experiment 2 significant differences were found in the ADV concentration 1 μg/ml (dominant animals > subdominant and subordinate animals, $P < 0.05$) and in 5 μg/ml (dominant > subordinate animals, $P < 0.05$) (Figure 1.2).

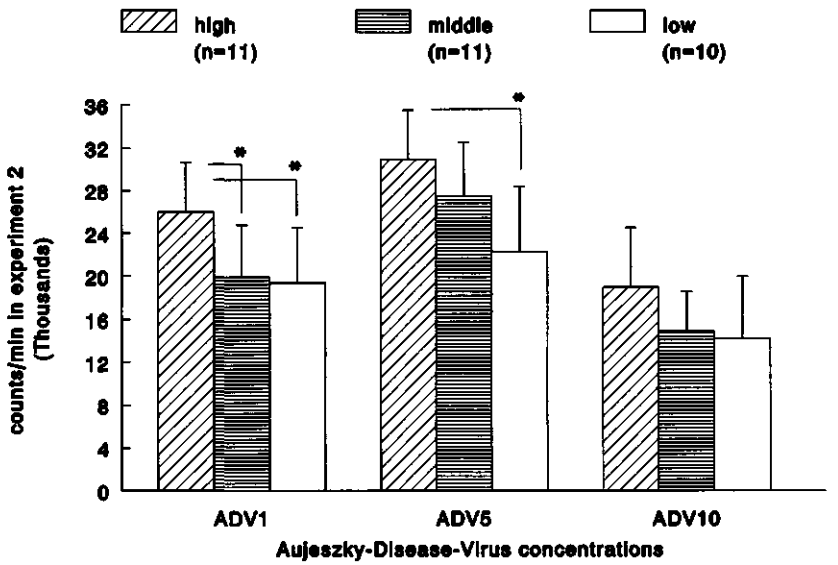

Figure 1.2. The mean number (± SEM) of counts/minute (cpm) in the ADV-specific lymphocyte stimulation test, using different Aujeszky virus concentrations (1, 5 and 10 μg/ml), minus the cpm of the control cultures (background counts) of high, middle and low ranking animals in experiment 2 (* = $P < 0.05$). Background cpm (± SEM) of high, middle and low ranking animals were, respectively, 1890 ± 396, 1546 ± 294 and 1086 ± 175.

In both experiments there were no significant differences in the ADV-specific LST between subdominant and subordinate pigs. Pearson's correlations showed in general that the higher the social rank of the pig the higher the cpm in the ADV-specific LST (Table 1.3).

Haematological and clinicochemical blood parameters

Before the ADV-challenge only minor differences existed in these variables between pigs of different social rank. In experiment 1 high ranking pigs had a lower total number of lymphocytes ($r_p(18) = - 0.43$, $P < 0.05$), and in experiment 2 a somewhat higher amount of α-globulin ($r_p(90) = + 0.24$, $P < 0.05$) than subdominant and subordinate ones. All other blood variables before the ADV-challenge did not significantly correlate with social status of the pigs. However,

four weeks after the ADV-challenge in both experiments low ranking pigs had a higher amount of total protein, α- and γ-globulin, and a higher total number of leucocytes and neutrophils (Table 1.3). Significant correlations between these blood variables and the social status of the individual pig decreases after Kendall's partial correlation analysis, using morbidity as a constant variable (range; $r_p(83) = -0.08/-0.12$; NS). This suggests that changes in these blood parameters is associated with morbidity and not with social status.

Table 1.3. Relations between individual social status and the ADV lymphocyte stimulation test using different ADV virus concentrations (1, 5 and 10μg/ml), and haematological and clinico-chemical blood variables four weeks after the ADV-challenge.

	Experiment 1	Experiment 2
ADV1	$r_p(12) = +0.64$ **	$r_p(32) = +0.35$ *
ADV5	$r_p(12) = +0.69$ **	$r_p(32) = +0.38$ *
ADV10	$r_p(12) = +0.69$ **	$r_p(32) = +0.25$ ns
Total protein	$r_p(14) = -0.47$ *	$r_p(82) = -0.26$ *
γ-globulin	$r_p(14) = -0.60$ **	$r_p(82) = -0.25$ *
α-globulin	$r_p(14) = -0.57$ **	$r_p(82) = -0.21$ *
Total leucocytes	$r_p(14) = -0.54$ *	$r_p(82) = -0.13$ ns
Lymphocytes	$r_p(14) = -0.29$ ns	$r_p(82) = -0.07$ ns
Neutrophils	$r_p(14) = -0.53$ *	$r_p(82) = -0.21$ *

* = $P < 0.05$; ** = $P < 0.01$; ns = not significant.

MHC-typing

A total of 15 different SLA-class I haplotypes and 13 different SLA-class II haplotypes were detected in the pigs (n=90) of experiment 2. Not all these different haplotypes were equally distributed among pigs of different social status. More high ranking pigs had allele HF1- (SLA-class I) ($\chi^2 = 8.50$, $P < 0.05$), and especially haplotype HF1-HF16 (8/27 = 29.6%) ($\chi^2 = 13.16$, $P < 0.01$) than middle- (2/32 = 6.3%) or low ranking pigs (0/31 = 0%). In contrast, more low ranking animals (14/31 = 45.2%) had either SLA-class II haplotype DF4-DF1, DF4-DF4 or DF4-DF7 ($\chi^2 = 9.13$, $P < 0.01$), than middle (8/32 = 25%) or high ranking pigs (3/27 = 11.1%). Besides that, no significant differences were found in the distribution of the SLA-class I and II haplotypes among pigs of different social status.

DISCUSSION

In intensive pig husbandry high levels of social stress are likely, since the animals involved are housed in prolonged confinement, in high density, and in homogeneous (by age and weight) groups from which it is impossible to escape. Under such conditions development and maintenance of a stable social structure will cause much stress (Meese and Ewbank, 1972). The present study showed substantial agreement between the social status as determined by ranking fights and the food competition tests. Moreover, high agreement in the outcome of the pairwise combinations of successive food competition tests was found. We, therefore, conclude that pigs in our experiments developed a stable social structure. Pigs could be divided in three groups per pen consisting of respectively dominant, subdominant and subordinate pigs. The individual social status of each pig was influenced by birth weight but not by sex. The heavier piglets at birth, male or female, take the higher social positions in their group, which agrees with other reports (McBride et al., 1964; Meese and Ewbank, 1972).

Results of the Aujeszky disease virus (ADV) challenge in both experiments demonstrated that dominant pigs were less susceptible for the Aujeszky virus than subdominant and subordinate ones. Although, mortality was somewhat higher in experiment 1 than in experiment 2; both experiments showed that this mortality was higher among subordinates than among subdominant and dominant ones. Results about morbidity were particularly striking because some pigs developed severe clinical signs while others remained clinically healthy. Nonetheless, more subordinate pigs showed these signs which were also more severe than for subdominant and dominant ones. Comparable data of a clear relation between position in a social hierarchy and disease susceptibility have not been widely described in the literature. Ebbesen et al. (1991) found that subordinate male mice had a higher incidence of virus induced leukaemia compared to dominant ones. But most studies reported different immune (Beden and Brain, 1985; Koolhaas and Bohus, 1989; Raab et al., 1986) and neuroendocrine (Ely and Henry, 1978; Fleshner et al., 1989; Von Holst, 1986) reactivity between individuals of different social rank, **suggesting** a relation between social status and disease susceptibility.

Kendall's correlations confirmed that morbidity caused high values of haematological and clinicochemical variables among subordinate pigs after the ADV-challenge. These findings suggest that such blood parameters may give valuable information about the health status of animals and can be of help in the clinical diagnosis of diseases as has been argued by others (Elbers et al., 1991; Unshelm, 1983). Higher levels of neutrophils and α-globulins may indicate chronic infections (Imlah and McTaggert, 1977), whereas elevated levels of γ-globulins reflect an

increased antibody production against an infectious agent like the Aujeszky virus (Taylor, 1984).

A high Aujeszky antibody production was observed in subordinate pigs after the ADV-challenge in experiment 1, but not in experiment 2. An important question is in how far significant relationships between social rank and immune capability in fact demonstrated that higher ranking pigs may have received more maternal derived antibodies (MDA) against the Aujeszky virus and in this way may be better protected than lower ranking pigs. This possibility is unlikely since variation in MDA prior to the ADV-challenge in both experiments was not related with the social status of the pig. Furthermore, regression analysis demonstrated that also variables as teat order, body weight and litter were not relevant in explaining MDA variation. Some reports demonstrated protective effects of MDA in susceptibility for ADV (Wittmann et al., 1979), while other ADV-experiments did not find these (de Leeuw et al., 1982). Hence, the significance of humoral immunity against an Aujeszky virus remains unclear and, as for most virus infections, is the cell-mediated immune response more important.

In both experiments the specific lymphocyte stimulation test showed that this cell-mediated immunity (CMI) was higher for dominant pigs than for subdominant and subordinate ones. Low virus susceptibility seemed to be related with high CMI, as has been argued also by Mason (1991). Therefore, pigs of high social status and associated high CMI were better protected against ADV.

Several authors emphasized that differences in immune reactivity and protection against diseases may be determined partially by genetics (Van der Zijp and Egberts, 1989; Zinkernagel and Doherty, 1979). MHC-typing in experiment 2 revealed that certain SLA-haplotypes, both class I and II, were not equally distributed among pigs of different social position; this suggests that the social status of a pig in a social hierarchy may be partially genetically determined. However, variability in SLA haplotypes was great and the number of pigs was too small to clarify possible causal relationships between genetic constitution, disease susceptibility and social rank of the individual pig.

In conclusion, the present data show that among domesticated group-housed pigs in a stable social structure large individual differences exist in immune reactivity and disease susceptibility. These differences appear to be related with the social status of the individual pig in the group. The higher disease resistance of dominant pigs against ADV may be associated with different neuroendocrine mechanisms, since several studies indicate rank-associated differences in neuroendocrine mechanisms (Ely and Henry, 1978; Von Holst, 1986). Besides that, human (McCann and Matthews, 1988) and animal studies (Benus et al., 1987; Von Holst, 1986) demonstrate consistent individual different behavioural strategies to stressors. These strategies

could well be the basis for different physiological, immunological and neuroendocrine programs leading to individual differences in disease susceptibility and stress pathologies. Therefore, further research will be focused on the individual strategies to stressors.

ACKNOWLEDGEMENTS

We thank; M.F. Sanders, D. van Gennip and A. Arriëns of the Department of Infectious Diseases and Immunology, Faculty of Veterinary, State University of Utrecht for performing the specific Lymphocyte Stimulation Test with Aujeszky-antigen and the SLA-typing class I and II with iso-electric focusing technique. Els Daniëls participated in the experiments and did extensive practical work. Haematological and chemical blood parameters were determined by the personnel of the laboratory of the Animal Health Service in South-Netherlands, under supervision of Ria Kroon. Henk Parmentier of the Department of Animal Husbandry, section Immunology of the Agricultural University of Wageningen made valuable comments on earlier drafts of the manuscript.

Chapter 2

INDIVIDUAL BEHAVIOURAL CHARACTERISTICS IN PIGS

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Published in: Applied Animal Behaviour Science 37:285-295 (1993)

INDIVIDUAL BEHAVIOURAL CHARACTERISTICS IN PIGS

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ABSTRACT

In two identical experiments a total of 218 piglets from 20 sows were used to test if consistent individual behavioural differences exist among pigs. At an age of 1-2 weeks piglets were divided between aggressive and non-aggressive individuals on the basis of their behaviour in two successive social confrontation tests (SC1 and SC2). Substantial agreement in this classification existed between the two observers and between SC1 and SC2. No significant gender and litter effects were found in the occurrences of aggressive behaviour. After mixing at 10 and again at 15 weeks of age, aggressive behaviour was mainly shown by the aggressive individuals as classified in the social confrontation tests. In a non-social backtest piglets, restrained in a supine position, were classified as resistant (R; > two escape attempts), intermediate (I; two escape attempts) or non-resistant (NR; < two escape attempts). Based on the outcome of these five successive backtests in weeks 1, 2 and 3 piglets were classified eventually as R (n=95), NR (n=77) or Doubtful (D; n=46). R piglets had a shorter latency to first resistance, but a higher number of vocalizations than NR ones, while the behaviour of the D piglets was in between. Piglets classified as R in the backtest were mostly the aggressive individuals, while NR piglets were mostly the non-aggressive ones; D piglets were equally distributed over aggressive and non-aggressive individuals. This association in behaviour and its consistency over time strongly suggests the existence of behavioural strategies to cope with conflict situations, that are typical of individual pigs and are measurable already in the very first weeks of their life.

Keywords: Pigs; Aggression; Social behaviour

INTRODUCTION

Intraspecific variability in animals' behaviour has long been neglected and mainly treated as 'noise' in the system (Slater, 1981). However, the high variation in for example feeding (Slater, 1974), sexual (Dunbar, 1983) and aggressive behaviour (Benus et al., 1987; Van Oortmerssen et al, 1985) in a population presumably presents more than a pure noise phenomenon. Animal studies have shown that basically two different strategies may co-exist in a population (Benus et al., 1987; Bohus et al., 1987; Slater, 1981). Both are equally successful depending on the stability or variability of the environment (Benus et al., 1990; Van Oortmerssen et al., 1985). The balance in a population between these two different strategies seemed to be realized by some

individuals adopting one strategy while the remainder adopts the other one (Slater, 1981). The existence of individual differences in behaviour may also be important in understanding the complex social relations among animals living in social groups. Studies in rodents revealed that individual behavioural strategy determines the eventually acquired social position of that individual in a social structure (Koolhaas and Bohus, 1989). The natural distribution of types of animals in a stable social structure may be not arbitrary. Therefore, this natural distribution of type of animals could be a guideline in the way we keep our animals in groups. For instance, putting only dominant animals in one group is asking for trouble. A mixture of dominant and subordinate ones may be more appropriate. If such a reasoning makes sense it might be worthwhile to compose groups of pigs in intensive husbandry on better grounds than is the case now. Therefore, we started to find out if individual behavioural strategies in pigs exist. For practical purpose it is essential that distinction between individual pigs is easy to establish and can be observed at an early age, and furthermore that such a distinction is consistent over time. The present study aims at these aspects.

MATERIAL AND METHODS

Housing and subjects

In two identical experiments (replicates) 218 pigs of 20 conventional sows (Yorkshire x Danish Landrace) were used. Sows farrowed within seven days. Farrowing crates measured 4.1 m^2. Sows were fed a commercial diet and had free access to water. Each piglet was permanently identified by an ear tattoo number. Male piglets were castrated three days after birth. Piglets had water and food *ad libitum* from day 7 on. All pigs were weaned, relocated and mixed at an age of 30 days and housed in pens of 7.7 m^2, with 10 (experiment 1) or 11 pigs (experiment 2) each. At an age of 10 and again at 15 weeks pigs were relocated and mixed and housed in large pens (4.6 m x 6.0 m) with 20 (experiment 1) or 22 pigs (experiment 2) each. Pigs were slaughtered at a live weight of approximately 90 kg ($\pm$ 21 weeks of age). During the entire experiment artificial lights were on from 07.00 until 19.00.

Social confrontation test

When one week old, three piglets (or sometimes two) from one litter were placed in a metal crate (1.6 m x 1.6 m x 0.78 m) together with three piglets (or sometimes two) from another litter; all piglets experienced this confrontation once. Each pig could be recognized by a number

painted on its back. This social confrontation test (SC1) lasted for 30 minutes. During this 30 minute period the occurrences of the following behavioural elements were recorded per individual piglet: (1) sniffing; (2) threat; (3) head knock; (4) biting; (5) fighting; (6) chasing; (7) fleeing; (8) withdrawal; (9) passive and also who started a fight (fight initiative). Frequency of total aggressive behaviour is the sum of the frequencies of the separate behavioural elements 2,3,4,5 and 6. Immediately after each observation two observers classified each piglet by qualitative impression as either an aggressive (A) or a non-aggressive (NA) individual. The SC1 was repeated one week latter (SC2) and all animals were classified anew using the same rules.

Backtest

In this test each piglet was individually put on its back and restrained in this supine position for sixty seconds while one hand was placed loosely over the head of the pig. During the suckling period the backtest was carried out five times; once in the first week and twice in the second and third week. In each of the five backtests a piglet making more than two escape attempts was classified as a resistant (R) individual and as a non-resistant (NR) when it made less than two escape attempts. When a piglet made exactly two escape attempts it was classified as an intermediate one. Moreover, the latency to first resistance and the number of vocalizations were noted down during each backtest. Overall classification of each individual piglet was based on the outcome of all 5 backtests. To be classified as a R individual it should have been at least 3 times a R outcome and not more than one time a NR outcome. Comparable rules were used for the classification of a NR individual. All remaining piglets were classified as doubtful (D).

Behaviour immediately after mixing

Immediately after mixing and relocation at 10 weeks of age two observers recorded for 30 minutes the occurrences of the same behavioural elements as in the social confrontation tests. These direct behavioural observations were made in two large pens (27.6 m^2) with 20 (experiment 1) or 22 pigs each (experiment 2). These large groups consisted of familiar and unfamiliar pigs. The behaviour of 79 selected pigs stemming from both experiments was analyzed in some more detail. Pigs could be recognized by a number painted on their back. The same procedure was repeated when the pigs were 15 weeks of age.

Statistical analysis

Data were analyzed using the statistical package for social science (SPSS-PC+) version 3.1 (Norusis, 1989). Frequencies of behavioural elements were checked for normal distribution

using the Examine procedure (Norusis, 1989). Kappa statistics are given as a measure of association between the two observers in classification by qualitative impression in aggressive and non-aggressive individuals in the two social confrontation tests (Kelsey et al., 1986; Seigel et al., 1992). Wilcoxon test was used for differences in behaviour elements between social confrontation test 1 and 2 (Siegel and Castellan, 1988). Regression models were used with polynomials of degree 3 for the analysis of the relationship between the classification in aggressive and non-aggressive individuals by qualitative impression and the quantitative occurrences of total aggressive behaviour (Norusis, 1989). Statistical differences between the regression equation of SC1 and SC2 were simultaneously tested using a "dummy" variable in the regression analysis (Draper and Smith, 1981). Cumulative percentages of agreement between successive backtests are given (Sackett, 1976). Differences in latency to first resistance and number of vocalizations in the backtests between R, NR and D individuals were analyzed with Tukey's multiple range test (Norusis, 1989). Kappa statistics are again given as a measure of test agreement between the social confrontation tests and the overall classification in the backtest. Differences in social behaviour elements directly after mixing at 10 and 15 weeks between aggressive and non-aggressive pigs as classified by qualitative impression in the social confrontation tests were analyzed with the Cross tabulation method, which measures a Chi-square (Norusis, 1989).

RESULTS

Data analysis showed comparable results for the two identical experiments and, therefore, both were combined for further analysis.

Social confrontation test

The classification in aggressive (A) and non-aggressive piglets (NA) in SC1 and SC2 based on qualitative impression by the two observers corresponded well with the quantitative distribution of aggressive behaviour (Figure 2.1). Furthermore, a substantial agreement existed between the two observers in their classification in A and NA piglets (SC1: $K = 0.93$; $P < 0.001$ and SC2: $K = 0.92$; $P < 0.001$). When a piglet showed no or only a single aggressive behaviour it was in all cases classified by the two observers as a NA individual, while when showing more than ten aggressive behavioural elements all piglets had been classified as an A individual. Classification in A or NA individuals varied between two and ten aggressive acts, depending on the overall aggressiveness of the SC-group (Figure 2.1).

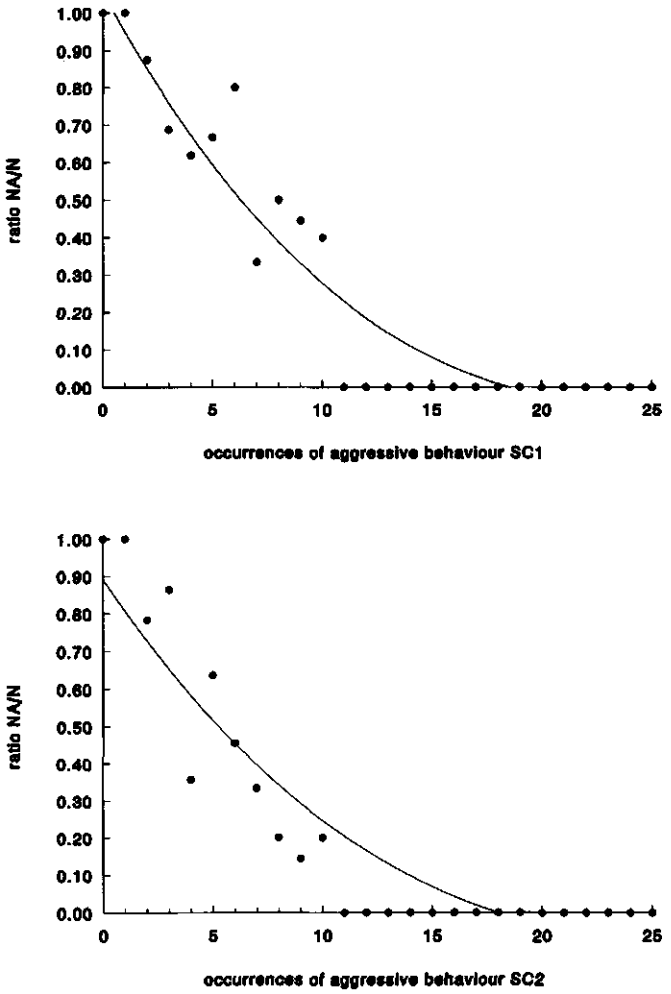

Figure 2.1. Comparison between classification in aggressive and non-aggressive piglets by qualitative impression and frequency of total aggressive behaviour in SC1 and SC2. Classification by qualitative impression is illustrated as the ratio of non-aggressive individuals (NA) of the total number of piglets (N): ratio NA/N.
Regression line SC1: $\hat{y} = 1.05 - 0.108x + 0.00337x^2 - 0.000033x^3 : R^2 = 0.96$.
Regression line SC2: $\hat{y} = 0.89 - 0.087x + 0.00245x^2 - 0.000021x^3 : R^2 = 0.94$.

Analysis of the regression coefficient of the dummy variable ($\beta = -0.05$; $P > 0.70$) revealed that the regression equation in SC1 and in SC2 was the same, indicating that qualitative impression and quantitative occurrences of aggressive behaviour coincide in both SC tests.

Moreover, classification in A or NA individuals was consistent over time because 84% of the piglets (K = 0.68; P < 0.001) were classified similarly in SC1 and SC2. A and NA piglets were equally distributed in SC1 and SC2 and between gender (SC1; 101 A (54♂/47♀) and 117 NA piglets (53♂/64♀): SC2; 111 A (57♂/54♀) and 107 NA ones (49♂/58♀). Moreover, no significant differences existed in the distribution of aggressive and non-aggressive piglets among litters (data not shown). Comparison of aggressive behaviour directed to litter- or non-littermates showed that the occurrences of total aggressive behaviour between littermates was less in SC2 than in SC1 (Z_S = -2.08; P <0.05); this was mainly dued to the reduced occurrences of biting (Z_S = -2.18; P < 0.05) and chasing (Z_S = -1.80; P < 0.05). In contrast chasing non-littermates was higher in SC2 than in SC1 (Z_S = 1.80; P < 0.05). In reaction to aggression, withdrawal behaviour decreased (Z_S = -1.71; P < 0.05), while flight behaviour increased (Z_S = 1.96; P < 0.05) in SC2 compared to SC1.

Backtest

The cumulative percentage of agreement as presented in table 2.1 indicated that 77.8 % of the piglets classified as R or NR in the first backtest were again R or NR in the second one. Furthermore 86.0% of the piglets classified as R or NR in the first two backtests were R or NR in the third one etcetera (Table 2.1). Piglets classified as intermediate ones (two escape attempts) in the first backtest had lower percentages of agreement in successive backtests (Table 2.1).

Table 2.1. Cumulative percentages of agreement in successive backtests of piglets classified as R or NR or as intermediate.

Successive Backtest	R/NR	Intermediate
1 → 2	77.8%	43.7%
1-2 → 3	86.0%	26.2%
1-2-3 → 4	95.3%	16.1%
1-2-3-4 → 5	98.0%	13.7%[a]

[a] 5 piglets showed in all 5 successive backtests two escape attempts.

Piglets were eventually classified as R, NR or doubtful (D) based on the outcome of all 5 successive backtests. The numbers of R, NR and D individuals were respectively; 95 (49♂/46♀), 77 (35♂/42♀) and 46 (23♂/23♀); gender was equally distributed among these groups. Moreover, no statistical differences were found in the distribution of R, NR and D piglets among litters (data

not shown). From all piglets eventually classified as R or NR 81.4% of them revealed this already in backtest one, 93% after two, 98.8% after three and 100% after four backtests.

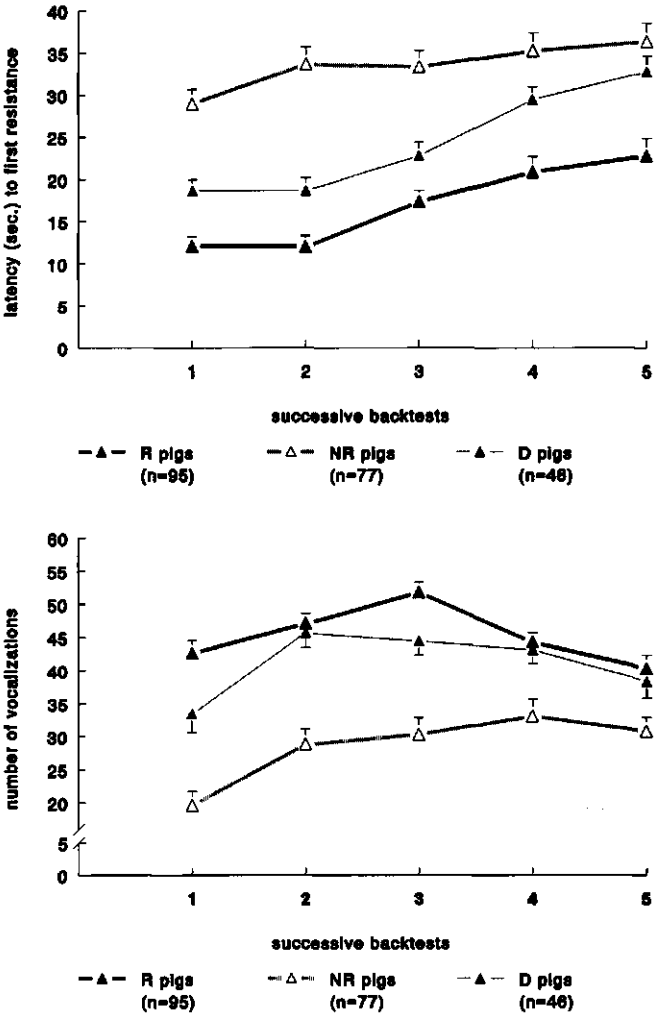

Figure 2.2. Latency (in sec.) to first resistance (above) and total number of vocalizations (below) in 5 successive backtests for resistant (R), doubtful (D) and non-resistant (NR) piglets.

Behaviour in the successive backtests revealed that latency to first resistance was lower, but number of vocalizations was higher for R piglets compared to NR ones. The D piglets showed an intermediate latency to first resistance and number of vocalizations compared to R and NR individuals (Figure 2.2).

Association between the SC- and the backtest

The measure of association between the SC- and the backtest showed that 74.4% (K = 0.49; $P < 0.01$) of the piglets classified as aggressive (A) or non-aggressive (NA) in SC1 and 75.6% in SC2 (K = 0.52; $P < 0.01$) were respectively the R and the NR individuals classified over all five successive backtests. This test agreement improved substantially (SC1: 84.4%, K = 0.66; $P < 0.001$ and SC2: 85.3%, K = 0.69; $P < 0.001$) when the same analysis was performed using only piglets that were classified in each of the five successive backtests as R or NR individuals (extremes). Piglets classified as D over the five backtests were equally distributed in A and NA individuals in both SC-tests (SC1: 21A / 25 NA and SC2: 26 A / 20 NA).

Behaviour directly after mixing

Pigs, after relocation to the large pens, first explored their new environment but aggressive interactions between unfamiliar pigs started within minutes. The aggressive pigs, classified by qualitative impression in the social confrontation tests, performed more aggressive behaviour, like biting ($P < 0.01$); initiating fights ($P < 0.01$); fighting back (10 weeks; $P < 0.01$; 15 weeks; not significant) and chasing a defeated conspecific ($P < 0.05$) in the 30 minutes observation period directly after mixing at 10 and 15 weeks of age than the non-aggressive pigs (Table 2.2).

Table 2.2. Behavioural elements per 30 minutes immediately after mixing at 10 and 15 weeks for A and NA pigs.

	10 weeks		15 weeks	
	A (n=41)	NA (n=38)	A (n=39)[1]	NA (n=38)
Behavioural elements				
Sniffing	6	3[2]	0	1
Head knock	7	5	3	0
Biting	168	43[a]	148	94[a]
Fights initiative	28	3[a]	44	9[a]
Fights back	28	4[a]	30	26
Chasing	40	18[b]	53	33[b]
Flight	41	58	49	71
Withdraw	47	36	30	34
Passive	2	2	0	1

[1] Two animals were removed from the group due to lameness.
[2] Total sum of frequencies / 30 min.
[a] $P < 0.01$; [b] $P < 0.05$.

DISCUSSION

The present study demonstrates that in pigs consistent individual behavioural characteristics exist. In a social setting, 1-2-week-old piglets could be divided into aggressive (A) and non-aggressive (NA) individuals. The classification of A or NA by qualitative impression highly correlated between observers and also corresponded nicely with quantitative parameters of aggressive behaviour. Therefore, by simply looking at the piglets behaviour in a social setting one gets a good impression of whether a piglet is an A or NA individual. Another important issue is the stability over time of the individual behavioural characteristics. Analysis showed that 84% of the piglets classified as A or NA in SC1 were again classified as A or NA in SC2. Moreover, the aggressive behavioural elements observed after mixing at 10 and 15 weeks of age were mainly shown by piglets that were classified as the aggressive ones in the SC tests shortly after birth, indicating that the behavioural response pattern of the individuals remained consistent over a long period of time despite different experiences.

In the non-social backtest piglets could be divided into individuals that resisted (R) or did not resisted (NR) this manipulation. Piglets that varied in their behaviour over the five successive backtests were classified as doubtful (D). These latter individuals need further attention, because it is still unclear whether these piglets represents some kind of intermediate group or are a result of 'noise' in the test. Nevertheless, most individuals could be classified as R or NR ones. The shorter latency to first resistance observed for the R piglets compared to NR ones was expected, because this increased the chance to be eventually classified as R. The higher number of vocalizations together with the escape attempts of the R piglets during the backtest could be an expression of trying to gain control over the aversive handling situation. The cumulative percentages of agreement of successive backtests indicated that piglets showed again a high consistency in their behavioural response. Furthermore most individuals, who were classified as R or NR based on five backtests, showed this already in the first or second test. Therefore, two backtests performed on piglets at an early age may suffice for practical use.

A surprisingly good association existed between the outcome of the backtest and of the SC test. The individuals that resisted in the backtests were the ones that were aggressive in a social situation, while the non-resistant individuals were the non-aggressive ones. A similar observation has been reported in other studies (Benus et al., 1990; Bohus et al., 1987). This association and its consistency over time strongly suggests rather general individual behavioural strategies to cope with conflict situations. The behaviour pattern of the aggressive and resistant piglet (A/R) reflects a more active behavioural response, while the non-aggressive and non-resistant one (NA/NR)

passive coping style. The resemblance of such different coping styles found in human (Glass, 1977) and animal studies (Benus et al., 1987, 1990; Corson and Corson, 1976; Von Holst, 1986) is striking. Further research must reveal how these different individual behavioural coping strategies are correlated with physiological and endocrine reactivity as has been put forward in other reports (Bohus et al., 1987).

In conclusion, the present data demonstrate consistent individual behavioural strategies in social and non-social situations in pigs. These different coping strategies may have great practical value. Pigs, living in social groups may disturb or support each other depending on their individual coping styles. Von Holst (1986) demonstrated, for instance, the stress reducing effects of social support in tree shrews. However, not all combinations may be equally successful; some animals do not match which may lead to increased stress. We have to investigate how in pigs an optimum group composition can be realized based on their individual characteristics. A matching combination of animals may stabilize the social structure in a group, which has a stress reducing effect beneficial for the farm animals as well as the farmer.

ACKNOWLEDGEMENTS

The authors wish to thank: the personnel of the pig farm of the National Institute of Animal Science, Research centre Foulum, Denmark for excellent care of the pigs. Paul Crauwels and Armin Elbers of the Animal Health Service in the Southern Netherlands for their statistical advice.

Chapter 3

INDIVIDUAL BEHAVIOURAL AND PHYSIOLOGICAL STRATEGIES IN PIGS

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Published in: Physiology & Behavior 55:39-46 (1994)

INDIVIDUAL BEHAVIOURAL AND PHYSIOLOGICAL STRATEGIES IN PIGS

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ABSTRACT

Previous experiments demonstrated consistent individual behavioural differences in pigs. Some showed a more active behavioural response (so-called A/R pigs) others a more passive behavioural response (so-called NA/NR pigs). In the present study we selected 32 A/R and 32 NA/NR individuals and tested them individually in an open field at three (OF1) and eight weeks of age (OF2). Individual response patterns were remarkably consistent between OF1 and OF2. While more A/R than NA/NR pigs made escape attempts, the A/R ones vocalized less, and were less inhibited to approach novel objects in OF1 and OF2 although they spent less time in exploring these objects than NA/NR pigs. Cortisol (CS) level after OF1 increased in A/R pigs but did not change in NA/NR ones, while CS level in OF2 remained constant in A/R pigs but decreased in NA/NR pigs. CS response to $ACTH_{1-39}$ was measured at three and eight weeks of age but did not differ between types. Basal CS level was higher in NA/NR than in A/R pigs and accompanied by adrenal hypertrophy. Mean heart rate (HR) was higher of A/R pigs compared to NA/NR ones in two backtests. HR of A/R pigs substantially increased (23.9 bpm = 15.5%) in reaction to the novel object in OF2, while HR of NA/NR ones only slightly increased (4.5 bpm = 2.9%), or even decreased (bradycardia). In addition, A/R pigs had more often heart deviations than NA/NR ones. The present study demonstrates that the two behavioural strategies of pigs are characterized by consistent differences in behavioural, physiological and endocrine responses to conflict situations.

Keywords: Coping behaviour; Open field; Cortisol; Heart rate.

INTRODUCTION

Variations in behavioural and physiological traits are considered as important biological individual characteristics to cope with relevant environmental changes that threaten homeostasis (Benus et al., 1987; Koolhaas and Benus, 1989; Levine and Wiener, 1989; Wiepkema and Schouten, 1988). Research has documented the existence of basically two different ways in coping with stressful situations (Benus et al., 1987; 1990; Bohus et al., 1987; Corson and Corson, 1976; Schouten and Wiepkema, 1991; Von Holst, 1986), namely: a) one in which the individual actively tries to remove the stressor or rapidly flees from it and b) one in which the individual freezes or gradually withdraws (Benus et al., 1987; 1990). Success of these coping strategies strongly depends on environmental conditions in that an active strategy appears to be more effective in a

stable environment, while the passive one is more suited under changing or unfamiliar environmental conditions (Benus et al., 1987). The interesting issue is that each individual prefers one or the other coping strategy. This preference is determined by genetic constitution and early life experiences (Suomi, 1987; Susman et al., 1989; Van Oortmerssen et al., 1985). Several studies have shown that in conflict situations the two behavioural strategies are highly correlated with different physiological and neuroendocrine mechanisms (Bohus et al., 1987; Sapolsky, 1990; Schouten and Wiepkema, 1991; Von Holst, 1986; Williams Jr. et al., 1982) including differential disease susceptibility and stress pathologies. All this implies that in stress research attention for the individual organism is crucial. Hessing et al. (1993) showed consistent behavioural differences between individual pigs. Since these differences were detectable already at an age of 1-2 weeks and, furthermore, remained consistent over time, this finding suggests stable individual strategies in pigs when coping. The present paper reports how these differences between pigs relate to different behavioural, physiological and endocrine responses under stress conditions.

MATERIAL AND METHODS

Animals and Housing

Subjects and housing have been described previously (Hessing et al., 1993). Briefly, in two identical experiments 218 pigs from 20 conventional sows (Yorkshire x Danish Landrace) were used. Male piglets were castrated three days after birth and each piglet was marked by an ear tattoo number. Pigs were weaned, relocated and mixed at an age of 30 days. At an age of 10 and 15 weeks all pigs were again mixed and relocated. During the suckling period all piglets were subjected to a social and non-social behavioural test. In two social confrontation tests piglets were classified as aggressive (A) or non-aggressive (NA) individuals. In the non-social backtest piglets were classified based on the outcome of their behaviour in five successive backtests into resistant (R), non-resistant (NR) or doubtful (D) individuals. Data presented elsewhere showed that of the 218 piglets; 95 were R, 77 were NR and 46 were D. A very good association existed between the outcome of the backtests and of the social confrontation tests, in that, the R pigs were mainly the A ones in the social confrontation tests (so-called A/R pigs), while the NR pigs were mainly the NA ones (so-called NA/NR pigs) (Hessing et al., 1993). In the present study, 16 A/R and 16 NA/NR pigs were selected in each replicate and these 64 pigs were observed in the following tests.

Open field test

At three weeks of age all 32 A/R and 32 NA/NR piglets were individually tested for 10 minutes in an open field (OF1) situated in a separate room. This OF measured 3 m x 3 m with 9 equal numbered squares and was surrounded by two walls and by two solid wooden fences (60 cm high). At eight weeks of age this test was repeated in an OF (OF2), which measured 5.5 m x 4.8 m with 20 numbered equal squares (1.1 m x 1.2 m) and surrounded by three walls and one solid hardboard fence (150 cm high). At the start of each test the animal was placed in the same start corner square in the OF. Each pig could be recognized by a number painted on its back and its behaviour was video-recorded during the entire 10 minutes period. Half way this period a novel object was introduced; in OF1 a cardboard box was thrown in the centre square and in OF2 a bucket was dropped from the ceiling down onto the floor and then lifted to a height of 15 centimetres above the floor. After the OF period the pig was transported back to its pen.

Behavioural response to novelty

In OF1 and OF2 behavioural responses were analyzed of the 32 A/R and the 32 NA/NR subjects for: (1) latency to leave first square (in sec.); (2) latency to reach centre square in OF1 or one of the two centre squares in OF2; (3) total time (sec.) in centre square(s); (4) number of line crossings (5) number of vocalizations; (6) number of pigs making contact with the novel object (7) latency to first contact with the novel object; (8) number of contacts with the novel object; (9) total contact time (sec.) with the novel object; (10) total time of exploration of the floor/walls; (11) number of pigs trying to escape and (12) number of pigs defecating. Behavioural parameters 7, 8 and 9 were calculated for only those pigs that touched the novel object within the observation period of 10 minutes.

Cortisol response

Cortisol response to novelty. Just before the start of OF1 and OF2 (t=0) a blood sample was taken from each individual pig. Because pigs were housed in groups they could not be cannulated and, therefore, blood samples had to be taken by vene puncture. Such a procedure causes much more stress than taking blood through a canulae. To minimize the influence of the blood sampling procedure on cortisol (CS) level the second blood sample was taken 90 minutes (t=90) after the start of each OF test. This design made it also possible to compare the results with the CS response to ACTH. Blood was preserved in heparin tubes on cold water (4°C) until centrifugation (400 x g for 10 minutes). Plasma aliquots were frozen at -20°C for later CS analysis. Plasma CS concentration in nmol/L was measured using the commercial Amerlite Cortisol Assay kit

(Amersham International plc, UK). According to information provided by the manufacturer (Amersham International plc, UK) sensitivity was 3 nmol/L, intra-assay coefficient of variation of 5.4% and inter-assay variation of 7.2%.

Cortisol response to ACTH. In all 64 selected pigs pituitary adrenal function was assessed at three and eight weeks of age. Each test was performed three days before the OF. Blood was taken (t=0) by vena puncture and thereafter 2.5 IU/kg live weight/pig of pure synthetic porcine adrenocorticotropic hormone ($ACTH_{1-39}$, 89 IU/mg; SIGMA®) was injected intramuscularly. A maximum time of 1 minute elapsed between catching the animal, completion of the first blood sample and the ACTH-injection. According to others (Hennesy et al., 1988; Shu-Hua et al., 1990; Von Borrell and Ladewig, 1987) pigs show a maximum CS response one hour after an injection of ACTH intravenously. As decribed above pigs were not cannulated and ACTH had to be injected intramuscularly. Because ACTH first has to diffuse into the bloodstream before reaching the adrenals the second blood sample was taken 90 minutes (t=90) later and a third one after 180 minutes (t=180) to investigate if CS level already returned to baseline level. Preservation of the blood and the CS analysis were as described above.

Cardiac response

Cardiac response in the backtest. At three weeks of age heart rate (HR) in beats per minute (bpm) was measured from the 16 A/R and 16 NA/NR piglets of experiment 2 during a backtest procedure (BT1). In this test each piglet was individually put on its back for two minutes, and at the same time HR was recorded with the Polar Sport Tester™ heart rate monitor of Polar electro OY®. Electrodes were gelled and HR interval capacity was set at 5 seconds; so 24 HR recordings were made for each subject. This procedure was repeated the next day (BT2).

Cardiac response to novelty. HR was also measured for each subject in OF2 of both experiments. A chest band was attached at the pig containing conductive gelled electrodes and the sensor/transmitter for HR measurements. On the back of the pig, attached to the chest band, was a small plastic box (8 x 4 x 2 cm) containing the HR monitor (Polar sport tester™). Since HR interval capacity was set at 5 seconds 120 HR recordings were made for each subject.

Pathological examination

At approximately 90 kg live weight (± 21 weeks of age) pigs were slaughtered. Lungs, liver, heart and stomach were macroscopically examined by a pathologist who was unaware of the identity of each subject. Lungs and liver alterations were scored as described elsewhere (Tielen et al., 1976). The heart was checked for pericarditis, congenital anomalies and the occurrence of

subepicardial and subendocardial lesions, inflammations, scar tissue and haemorrhages. Furthermore, heart and left ventricle were weighed. Stomach damage was scored as described elsewhere (Hessing et al., 1992). The adrenals were immediately weighed and thereafter frozen in liquid nitrogen and kept at -80°C for later microscopical analysis in which three slides of 25μm were made with the Kyrostat (1720 Leitz®) at the middle of the adrenal of each subject. These slides were stained as described elsewhere (Mallory, 1961) with slight modifications and surface areas of the cortex and medulla were measured by an applied image analyses system (Scan beam) (Henkel and Hassing, 1993).

Statistical analysis

Data were analyzed using the Statistical Package for Social Science (SPSS-PC+) version 3.1 (Norusis, 1989). Differences in frequencies/durations of the behavioural elements in OF1 and OF2 and mean HR in the backtests and OF2 were tested by normal analysis of variance (Norusis, 1989). Type of pig (A/R or NA/NR), litter and gender were used as factors; the factor gender was later erased from data analysis since it did not significantly contribute to the model. The number of A/R and NA/NR animals that tried to escape in OF1 or touched the bucket in OF2 was tested with the Chi^2-test (Siegel and Castellan Jr., 1988). Wilcoxon test was used for differences in behavioural elements between OF1 and OF2 (Siegel and Castellan Jr., 1988). A paired t-test was used to test the difference in plasma CS levels before (t=0) and after (t=90) OF1 and OF2. The CS response to the ACTH administration and the cardiac response to the novel object (bucket) in OF2 were tested with special contrasts in the repeated measurements analysis of variance (Norusis, 1989). The distribution of A/R and NA/NR pigs with pathological alterations were tested with the Chi^2 (Norusis, 1989). Differences in weight of the heart, left ventricle of the heart and adrenals (in % body weight) and the surface area of the adrenal-cortex and medulla between A/R and NA/NR pigs were again analyzed by normal variance analysis (Norusis, 1989).

RESULTS

Data analysis showed comparable results for the two identical experiments and, therefore, they were combined for further analysis.

Behavioural response to novelty

Each subject was individually transported to the OF and placed into the start corner square and stayed there for about 10 to 20 seconds before it started to move around. In OF1, piglets remained restless continuously by walking and running criss-cross through the OF and squealing frequently. Some of the piglets started jumping against the wooden fences trying to escape the OF. After the cardboard box was thrown into the OF piglets turned towards this novel object, but hesitated to approach and touch it. In OF2 behaviour was less agitated compared to OF1. Squealing did hardly occur and mainly short and long grunts were observed, pigs spent more time exploring the OF and they did not try to escape. After the bucket dropped onto the floor pigs again turned towards the novel object but not all of them dared touching it.

Differences in behavioural parameters of the 10 minute period in OF1 and OF2 of the 32 A/R and 32 NA/NR pigs are presented in table 3.1. In OF1, A/R pigs vocalized less than NA/NR ones ($F(1,63)=5.46$, $P < 0.05$) and data suggest that the escape attempts were performed more by A/R (16/32) than by NA/NR (9/32) subjects ($\chi^2 = 3.22$, $P < 0.10$). After the cardboard box was thrown into the OF latency to touch it was shorter for A/R than for NA/NR pigs ($F(1,61)= 6.23$, $P < 0.05$). However, after A/R pigs had approached and touched the box they were less interested in it than NA/NR ones ($F(1,61)=3.98$, $P < 0.05$) (Table 3.1). In OF2 the overall levels of some behavioural parameters differed significantly from the ones in OF1; vocalizations were much less frequent ($Z_s = -3.67$, $P < 0.01$), while latency to reach centre squares ($Z_s = 1.93$, $P < 0.05$), latency to touch the novel object ($Z_s = 3.45$, $P < 0.01$) and total time of exploration were much longer ($Z_s = 3.12$, $P < 0.05$) in OF2 in both types of pigs. Nevertheless, the behavioural differences between A/R and NA/NR subjects were comparable in OF2 and OF1 (Table 3.1). A/R pigs again vocalized less than NA/NR pigs ($F(1,63)=5.10$, $P < 0.05$). After the bucket dropped down onto the floor more A/R (25/32) than NA/NR (17/32) pigs touched it ($\chi^2 = 4.43$, $P < 0.05$) and the latency to touch it was again shortest for these A/R subjects ($F(1,41) =6.48$, $P < 0.05$). Moreover, the OF2 data suggest that these A/R pigs again were less interested in the bucket than the NA/NR ones ($F(1,41)=3.43$, $P < 0.10$) (Table 3.1). All other behavioural parameters in OF1 and OF2 were not significantly different between A/R and NA/NR individuals; furthermore, no significant litter-effects were found (data not shown).

Table 3.1. Behavioural parameters of the 32 A/R and the 32 NA/NR pigs in open field (OF) 1 and OF2. (1=latency to leave first square (sec.); 2=latency to reach centre square(s); 3=total time in centre square(s); 4=number of line crossings; 5=vocalizations; 6=number of pigs touching the novel object; 7=latency to first contact with novel object; 8=number of contacts with the novel object; 9=total contact time with the novel object; 10=total exploration time; 11=number of pigs making escape attempts; 12=number of pigs defecating).

Behavioural elements	OF1		OF2	
	A/R pigs	NA/NR pigs	A/R pigs	NA/NR pigs
1	14.4 ± 2.8	18.1 ± 3.3	11.5 ± 1.9	14.1 ± 3.8
2	36.6 ± 6.2	40.6 ± 8.2	56.7 ± 5.3	67.0 ± 9.9
3	61.8 ± 8.0	62.8 ± 6.0	76.3 ± 6.7	78.5 ± 6.1
4	84.9 ± 6.1	83.0 ± 5.8	77.5 ± 6.0	80.0 ± 7.9
5	369.0 ± 30.1	450.2 ± 31.2[b]	119.2 ± 15.7	184.0 ± 24.0[b]
6	31/32 (96.9%)	31/32 (96.9%)	25/32 (78.1%)	17/32 (53.1%)[e]
7	15.2 ± 2.1	25.6 ± 4.3[c]	81.9 ± 10.3	113.2 ± 18.6[c]
8	14.8 ± 1.2	14.7 ± 1.1	5.4 ± 1.0	7.5 ± 2.1
9	29.1 ± 2.9	39.7 ± 3.1[b]	20.8 ± 3.7	30.7 ± 4.6[a]
10	112.4 ± 7.1	124.6 ± 8.6	210.9 ± 24.1	228.6 ± 21.5
11	16/32 (50.0%)	9/32 (28.1%)[d]	0/32 (0.0%)	0/32 (0.0%)
12	15/32 (46.9%)	12/32 (37.5%)	18/32 (56.3%)	15/32 (46.9%)

[a] $P < 0.10$; [b] $P < 0.05$; [c] $P < 0.01$; [d] $\chi^2 = 3.22$; $P < 0.10$; [e] $\chi^2 = 4.43$; $P < 0.05$

Cortisol response

Cortisol response to novelty. In OF1, NA/NR piglets had a higher basal plasma cortisol (CS) level (mean=84.8 nmol/L, SEM=8.31) than A/R ones (mean=69.1 nmol/L, SEM=5.21), $F(1,63)=3.68$, $P < 0.05$. However, A/R piglets demonstrated a significant increase in CS level after OF1 (t=90) (mean=87.5 nmol/L, SEM=6.30; paired $t_{32} = 4.86$, $P < 0.05$), while this CS level of NA/NR piglets did not differ from the basal one (mean=87.9 nmol/L, SEM=8.62; paired $t_{32} = 0.63$, NS) (Figure 3.1). In OF2, basal plasma CS levels were significantly ($P < 0.05$) higher than the ones in OF1, while within OF2 these levels tended to be higher for NA/NR pigs (mean=103.7 nmol/L, SEM=5.69) than for A/R ones (mean=90.8 nmol/L, SEM=6.53), $F(1,63)=2.90$, $P < 0.10$. Plasma CS levels after OF2 (t=90) surprisingly decreased for NA/NR pigs (mean=78.2 nmol/L, SEM=5.81; paired $t_{32} = 4.00$, $P < 0.05$), while plasma CS levels of A/R pigs after OF2 did not differ from basal ones (mean=93.3 nmol/L, SEM=7.62; paired $t_{32} = -0.29$, NS) (Figure 3.1).

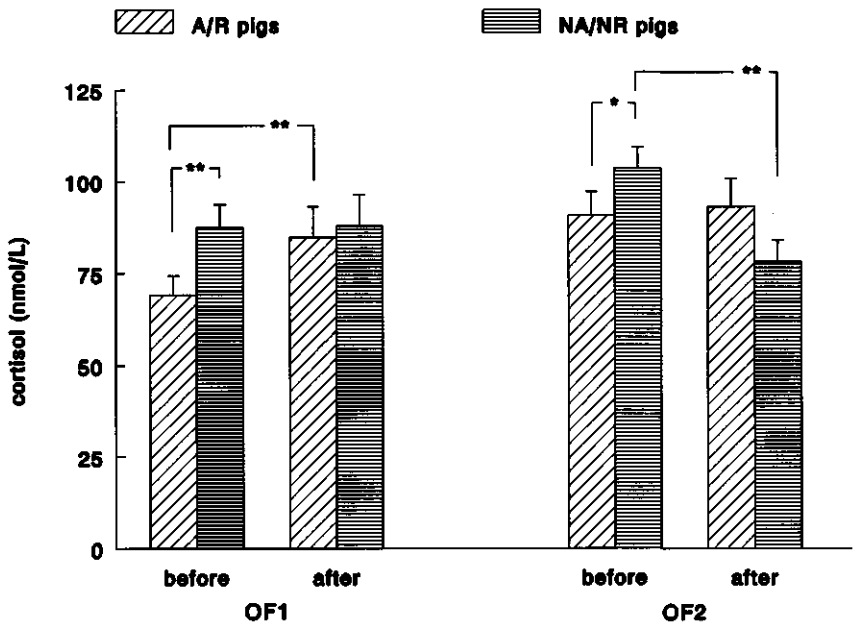

Figure 3.1. Plasma cortisol (nmol/L) level of 32 A/R and 32 NA/NR pigs before (t=0) and 90 minutes (t=90) after OF1 and OF2. (* = $P < 0.10$; ** = $P < 0.05$)

Cortisol response to ACTH. Basal plasma CS levels (t=0) at three weeks were higher for NA/NR piglets (mean=97.0 nmol/L, SEM=6.90) compared to A/R ones (mean=77.5 nmol/L, SEM=4.91), $F(1,63)=13.39$, $P < 0.01$, and tended to be higher for NA/NR subjects at eight weeks (mean=122.7 nmol/L, SEM=6.01) than for A/R pigs (mean=108.0 nmol/L, SEM=7.10), $F(1,63)=2.72$, $P < 0.10$. (Figure 3.2). Moreover, at eight weeks this basal plasma CS was higher than at three weeks both for A/R and NA/NR pigs, $F(1,63)=23.48$, $P < 0.001$. The plasma CS response to ACTH at three weeks did not differ significantly from the CS response to ACTH at eight weeks, ($F(1,63)=2.03$, NS); moreover, it was similar for A/R and NA/NR pigs (three weeks: $F(1,63)=1.92$, NS; eight weeks: $F(1,63)=0.02$, NS). Both types showed a strong increase in plasma CS level at t=90 and thereafter a strong decrease at t=180 (three weeks: $F(1,63)=115.49$, $P < 0.001$; eight weeks: $F(1,63)=208.75$, $P < 0.001$). The plasma CS levels at t=180 were significantly lower than basal plasma CS levels (t=0) for A/R and NA/NR pigs at three ($F(1,63)=4.44$, $P < 0.05$) and at eight weeks ($F(1,63)=87.90$, $P < 0.001$).

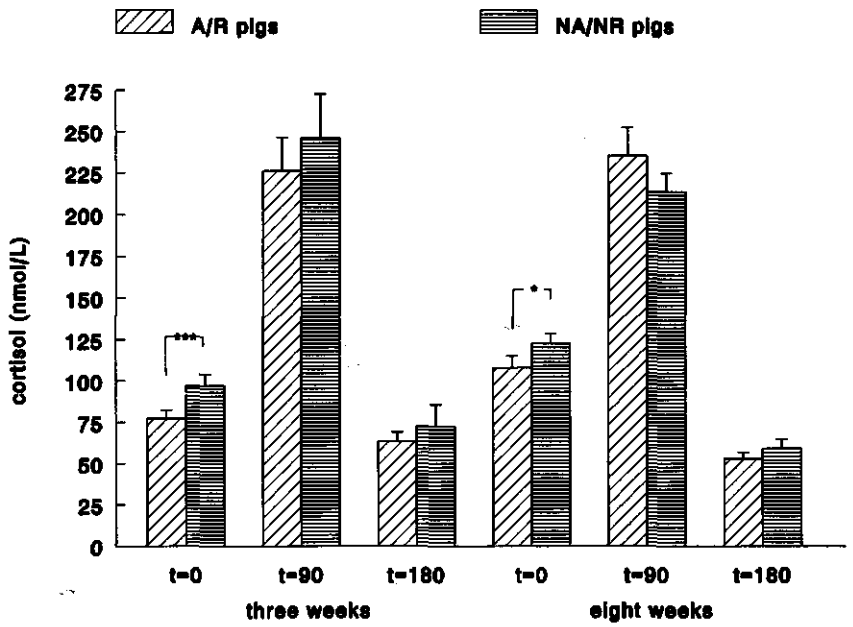

Figure 3.2. Plasma cortisol (nmol/L) levels of 32 A/R and 32 NA/NR pigs before (t=0), 90 minutes (t=90) after and 180 minutes (t=180) after $ACTH_{1-39}$ administration at three and eight weeks (* = $P < 0.10$; *** = $P < 0.01$).

Cardiac response

Cardiac responses in the backtests. Cardiac responses were similar for the two backtests in experiment 2. Therefore, only the mean heart rate (HR) changes are shown as they occurred in backtest 2 (BT2) (Figure 3.3). A/R piglets had a higher mean HR in backtest 1 (BT1) (mean= 165.4 bpm, SD=13.58) and in BT2 (mean=169.6 bpm, SD=18.51) than NA/NR ones (BT1: mean=153.9, SD=17.36; $F(1,31)=4.34$, $P < 0.05$ and BT2: mean=154.8, SD=21.17; $F(1,31) =4.42$, $P < 0.05$). However, the course of the cardiac response within BT1 and BT2 was the same for both types (BT1: time x type of pig interaction: $F(23,736)=0.62$, NS; BT2 (time x type of pig interaction: $F(23,736)=1.78$, NS) (Figure 3.3). Mean HR and cardiac response in BT1 and BT2 did not differ significantly among litters (data not shown).

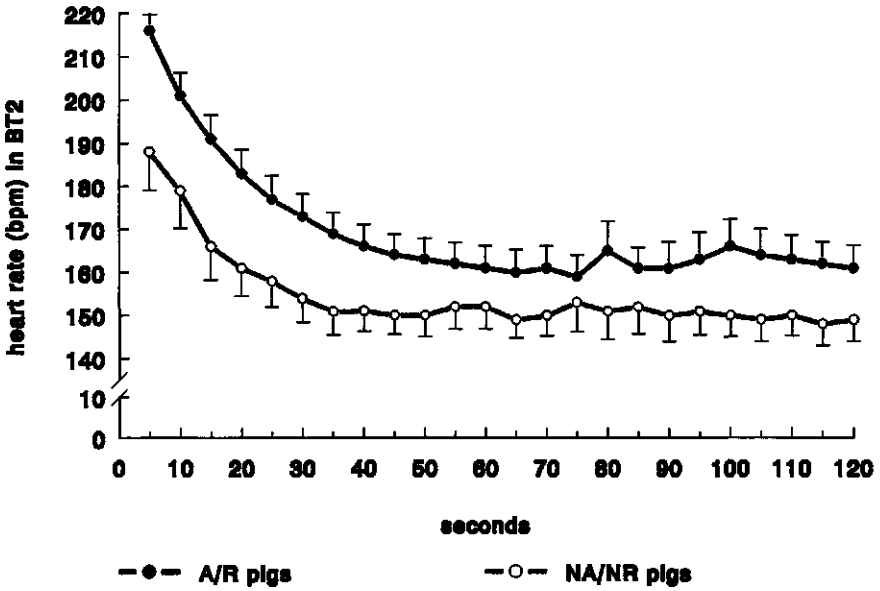

Figure 3.3. Cardiac response in backtest 2 (BT2) of the 16 A/R and the 16 NA/NR pigs in experiment 2.

Cardiac responses to novelty. One A/R pig and two NA/NR pigs were skipped from statistical analysis due to missing HR values, leaving 31 A/R and 30 NA/NR individuals. Mean HR in the first minute of the OF was highest in A/R pigs (mean=179.8 bpm, SD=16.10) compared to NA/NR ones (mean=166.1 bpm, SD=14.61), $F(1,60)=12.15$, $P < 0.01$. In the four minutes thereafter mean HR did not differ significantly between both types (A/R pigs; mean=153.9 bpm, SD=11.13 and NA/NR pigs; mean=153.0 bpm, SD=11.45, NS) (Figure 3.4). However, the cardiac response to the bucket markedly differed between A/R and NA/NR pigs. While the HR of A/R pigs increased substantially with an average of 23.9 bpm (15.5%, SD=13.12, $P < 0.001$), such a rise was non-significant in NA/NR pigs being only 4.5 bpm (2.9%, SD=25.18, NS) (Figure 3.4). Most surprisingly 10 of the 30 (33.3%) NA/NR pigs even demonstrated a significant HR decrease. Such marked differences in cardiac response to the bucket is illustrated for two representative A/R and NA/NR pigs (Figure 3.4). Cardiac response lasted approximately one minute and in the four minutes thereafter mean HR again did not differ significantly between both types (A/R pigs; mean=151.0 bpm, SD=13.31 and NA/NR pigs; mean=149.1 bpm, SD=15.88) (Figure 3.4).

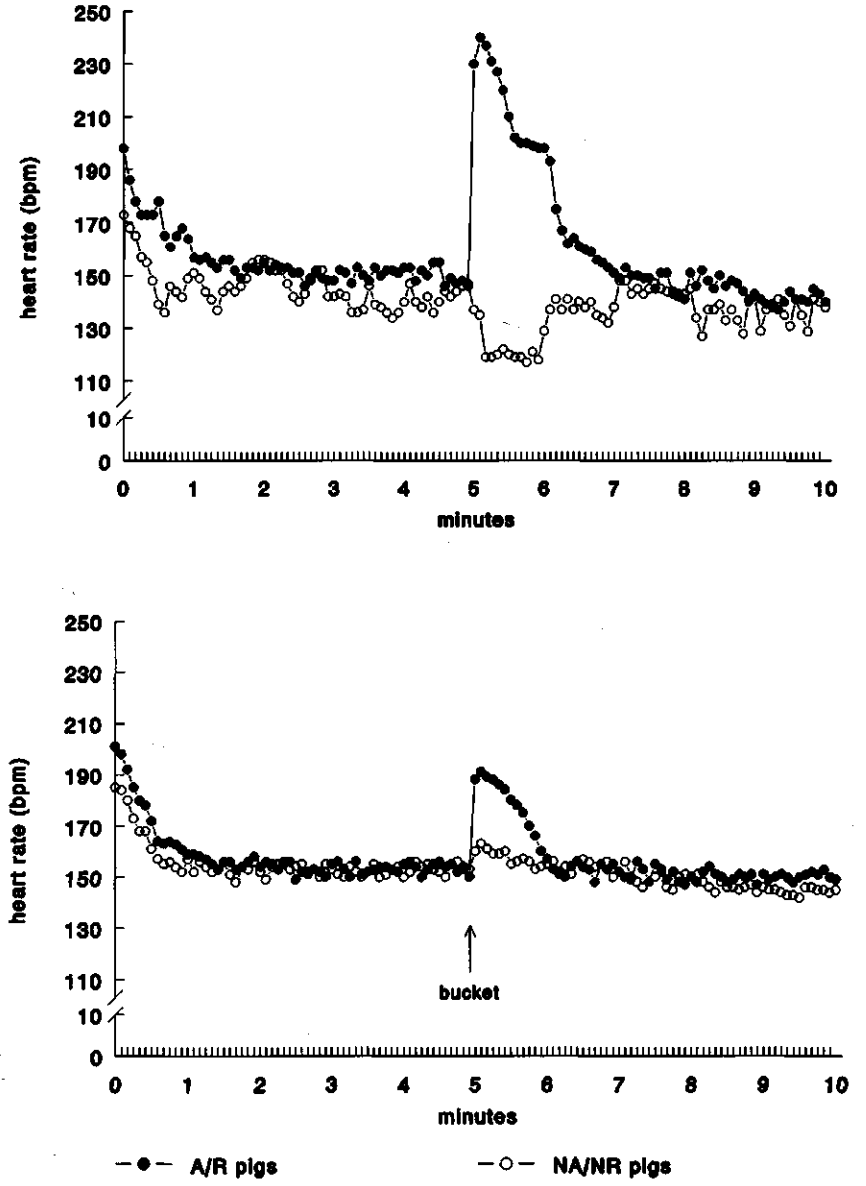

Figure 3.4. Cardiac response to novelty and to the novel object (bucket) in OF2 of A/R (n=31) and NA/NR pigs (*n*=30; below). Above: the same for one A/R and one NA/NR pig. (*t*=0; start of the open field test: t=5; bucket into the open field and t=10; end of the open field test).

Pathological examination

Pathological examination of the slaughtered pigs revealed 1 A/R (3.1%) and 1 NA/NR pig (3.1%) with abscesses in the lungs. Liver alterations were not found. In 65.6% (21/32) of the A/R pigs subepicardial and subendocardial haemorrhages in the heart muscle were found, while this was the case for only 34.4% (11/32) in the NA/NR pigs; this difference is significant (χ^2 = 6.25, $P < 0.05$), Two of these A/R pigs (6.2%) had also subepicardial scar tissue. Heart weight (in % body weight) did not differ significantly between A/R (mean=0.365%, SD=0.07) and NA/NR pigs (mean=0.357%, SD=0.04) and the same holds for weight of the left ventricle (respectively; mean=0.184%, SD=0.03 and mean=0.180%, SD=0.02). Although 7 A/R and 7 NA/NR pigs (=21.6%) had slight hyperkeratosis on the pars oesophagea of the stomach, stomach ulcerations were not found. Adrenals of the NA/NR pigs weighed (in % body weight x 10^{-3}) more (mean=2.43, SD=0.06) than those of A/R pigs (mean=2.12, SD=0.08), $F(1,63)=10.04$, $P < 0.01$. No significant differences were found in weight of the adrenals among litters ($F(19,63)=0.82$). Due to artifacts in the microscopical slides of adrenal tissue 29 A/R and 31 NA/NR adrenals could be analyzed. Cortex surface area did not differ significantly between A/R (mean=1833.0 mm^2, SEM=64.53) and NA/NR pigs (mean=1905.9 mm^2, SEM=77.35), and the same holds for medulla surface area of A/R (mean=516.5 mm^2, SEM=34.26) and of NA/NR pigs (mean=489.5 mm^2, SEM=38.25).

DISCUSSION

Results of previous experiments revealed consistent behavioural differences among individual pigs, that at an early age were classified as showing an active behavioural response (so-called A/R pigs) or a passive behavioural response (so-called NA/NR pigs) in social and non-social test situations (Hessing et al., 1993). Such individual different coping strategies (active or passive) have also been described for other species (Benus et al., 1990; Blumenthal, 1983; Koolhaas and Bohus, 1989; Mormède et al., 1984; Sapolsky, 1990; Slater, 1981; Von Holst, 1986). The present study showed that these individual coping styles in pigs are associated with consistent differences in behavioural, physiological and endocrine responses in stressful situations.

In OF1 three weeks old suckling piglets behaved very disturbed and squealed frequently; some of them tried even to escape energetically. In OF2 the eight weeks old and weaned pigs behaved much less agitated; they rarely squealed, they did not try to escape, and took more time to explore the OF. The different magnitude of behavioural responses in OF2 may have been

caused by the prior experience of OF1. However, it is more likely that in OF1 the social separation from penmates and especially from the sow caused dramatic stress for these young animals. In spite of the large emotional differences between the animals in OF1 and OF2 the distinction between the A/R and the NA/NR types remained similar. Since the tendency to escape was greatest in the A/R pigs, we conclude that these pigs, more than the NA/NR ones, are characterized by a so-called active behavioural strategy (Benus et al., 1987; 1990). A new element in the characterization of active and passive coping styles was the recorded difference in vocalizing. The NA/NR pigs have a significantly higher tendency to do so than the A/R pigs. A more detailed analysis of these vocalizations is needed before the observed difference can be discussed adequately. It is most interesting that the A/R pigs not only contacted a novel object sooner than the NA/NR pigs did, but that the subsequent contacts has a much shorter duration than those of the NA/NR animals. This difference suggests that the A/R pigs explored rapidly and superficially, while the NA/NR ones did so gradually but more intensively. This supports the idea that passive copers (the NA/NR animals) strongly attach to extrinsic stimuli that govern their behaviour (Benus et al., 1987).

Behavioural responses to novelty have widely been associated with fearfulness or level of excitement leading to increased pituitary adrenal (P-A) secretion (Hennesy and Levine, 1979; Levine and Wiener, 1989; Von Borrell and Ladewig, 1987). The present results showed that the cortisol (CS) response to novelty differed between both types of pigs; after OF1 CS level increased in A/R pigs but remained constant in NA/NR ones. This may suggest that A/R pigs had a more reactive CS response to novelty than NA/NR ones. But, we can also postulate that NA/NR pigs showed a much quicker and shorter CS response; for a final conclusion we need more data within the interval t=0-90. CS response to exogenous synthetic ACTH revealed no significant differences between the two types and contrasts the CS response to novelty. However, although the adrenocortical response to ACTH may reflect the response to a stressor (Hennessy et al., 1988; Sua-Hua et al., 1990), it remains highly questionable whether a pharmacological dose of ACTH as used here actually simulates relevant aspects of a natural stressor. Presumably we only measured the maximum capacity of CS production of the adrenals in reaction to an overdose of ACTH; this corresponds with the drop in CS below baseline level seen three hours after ACTH administration. As in other studies (Hennessy et al., 1988; Shu-Hua et al., 1990; Von Borrell and Ladewig, 1987) the CS response to ACTH was consistent over time. While the interpretation of the CS responses to novelty or ACTH remains somewhat speculative, the reported basal CS levels may be more informative. These levels were always higher in NA/NR pigs than in A/R pigs, which supports the hypothesis presented elsewhere that behavioural passive individuals have a

more active P-A system (Bohus et al., 1987; Henry and Stephens-Larson, 1985; Sapolsky, 1990; Von Holst, 1986). This corresponds with the adrenal hypertrophy observed in the NA/NR individuals. The higher weight of the NA/NR adrenals was, however, not accompanied by a significant enlargement of the adrenal cortex area. High basal CS levels may eventually lead to detrimental effects on health and immune reactivity (Kelley, 1985; Westly and Kelley, 1984); data on this point will be reported separately (Hessing et al., 1994b).

In the present study we also found consistent differences in cardiac responses between A/R and NA/NR pigs. Several reports have already demonstrated great differences in individual stress responses when subjects were challenged seriously (Pfister, 1979; Schouten and Wiepkema, 1991; Susman et al., 1989; Von Holst, 1986). Cardiac responses in the backtests and OF2 substantiate these findings. In the two backtests and in the first minute in OF2 A/R pigs had a higher mean heart rate (HR) than NA/NR ones. Moreover, after the bucket dropped onto the floor cardiac response showed remarkable differences between subjects; all A/R pigs demonstrated a HR increase (tachycardia), whereas one-third of the NA/NR pigs even showed a HR decrease (bradycardia). Tachycardia demonstrates an arousal of the sympathetic nervous system, while bradycardia suggests a vagal activation (Contrada et al., 1982; Fokkema et al., 1988; Glass, 1977). If so this implies that the A/R pigs reacted predominantly with a sympathetic stress response, whereas the NA/NR pigs performed a parasympathetic stress response; these findings strongly parallel human and animal data (Bohus et al., 1987; Blumenthal et al., 1983; Henry and Stephens-Larson, 1985). Although the sympathetic style of A/R pigs did not lead to hypertrophy of the heart many A/R pigs had haemorrhages on the heart muscle. The etiology and pathogenesis of these haemorrhages are not fully clear (Johansson et al., 1974; Jönnson and Johansson, 1974), although some studies on the influence of stress on pigs suggest a crucial role for catecholamines (Sordahl, 1986).

In conclusion, pigs demonstrate the existence of consistent individual behavioural, endocrine and physiological differences in the response to conflict situations leading to different stress pathologies. Active A/R pigs reacted more with a sympathetic nervous stress response leading to heart alterations, whereas behaviourally passive NA/NR pigs reacted more in a parasympathetic way, but had a more active P-A system leading to adrenal hypertrophy. These findings show that the different behavioural strategies of A/R and NA/NR pigs represent two different but presumably equally effective coping styles each having its own price.

ACKNOWLEDGEMENTS

We thank the personnel of the pig farm of the National Institute of Animal Science, Research centre Foulum, Denmark for excellent care of the pigs. Poul Henkel of the Research centre Foulum kindly provided the heart rate equipment and Lucia Lansbergen of the Agricultural University of Wageningen did extensive work on the microscopical slides of the adrenals.

Chapter 4

INDIVIDUAL DIFFERENCES IN CELL-MEDIATED AND HUMORAL IMMUNITY IN PIGS

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Accepted for publication in: Veterinary Immunology and Immunopathology

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ABSTRACT

Previous experiments displayed consistent individual behavioural differences in pigs. Some showed a more active behavioural response (so-called A/R pigs) others a more passive behavioural response (so-called NA/NR pigs). Moreover, these behavioural coping strategies were associated with different behavioural, physiological and endocrine responses under stress conditions. In the present study we selected 32 A/R and 32 NA/NR individuals and tested their immune reactivity in reaction to stress using several cell-mediated (CMI) and humoral immunological tests. Active A/R pigs had a higher in vivo and in vitro CMI to nonspecific and specific antigens. While after stress CMI was much more reduced in A/R than in NA/NR pigs. In contrast, humoral immunity was highest in NA/NR pigs. Furthermore, some serologically typed swine lymphocyte antigen (SLA) class I haplotypes were not equally distributed between A/R and NA/NR pigs. In general, these findings show that measurement of immune reactivity is an important tool to define how animals cope with environmental demands. Above all, it emphasizes that attention for the individual organism, in this, is crucial.

Keywords; Pigs; Coping Behaviour; Cell-mediated immunity; Humoral immunity

INTRODUCTION

To maintain homeostasis, vertebrates adapt to changes in internal and external milieu with a chain of behavioural, physiological, neuroendocrine and immunological responses regulated by the brain (Ader et al., 1991). Several studies have shown that individuals differ qualitatively in these adaptive responses when exposed to the same environmental changes (Bohus and Koolhaas, 1991; Dantzer and Mormède, 1983). These differences are considered as important individual coping characteristics (Benus et al., 1987; 1990). The data available suggest basically two different ways of coping, active or passive (Benus et al., 1987; 1990; Corson and Corson, 1976; Schouten and Wiepkema, 1991). The behaviourally active individuals try to remove the stressor or rapidly flee from it, whereas the behaviourally passive ones freeze and gradually withdraw (Benus et al., 1987). Both coping strategies are equally effective and have their own costs (Henry and Stephens-Larsson, 1985; Von Holst, 1986). Surprisingly, however, each individual seems to be

predisposed for one or the other coping style (Benus et al., 1987); this is determined by genetic constitution and early life experiences (Suomi, 1987; Van Oortmerssen et al., 1985). Previously, we showed the existence of consistent individual behavioural differences in pigs when coping (Hessing et al., 1993). As in other studies (Bohus et al., 1987; Fokkema et al., 1988) these differences were associated with different behavioural, physiological and endocrine responses under stress conditions (Hessing et al., 1994a). The differential involvement of the brain may also influence the communication between the brain and the immune system (Ader et al., 1991; Dantzer and Kelley, 1989), and this makes it plausible that the individual coping styles are also reflected in different immune parameters. The present work reports about individual differences in cell-mediated and humoral immunity as related to different coping styles.

MATERIAL AND METHODS

Subjects

In two identical experiments 218 pigs from 20 conventional sows (Yorkshire x Danish Landrace) were used. The sows farrowed within seven days. Male piglets were castrated three days after birth and each piglet was marked by an ear tattoo number. Piglets had *ad libitum* feed and water from day 7 on. During the suckling period all piglets were subjected to a social and non-social behavioral test as described previously (Hessing et al., 1993). Briefly, in two social confrontation tests piglets were classified as aggressive (A) or non-aggressive (NA) individuals. In five successive non-social backtests piglets were classified as a resistant (R), a non-resistant (NR) or a doubtful (D) individual based on the number of escape attempts. Data presented in detail elsewhere (Hessing et al., 1993) showed that of the 218 piglets; 95 were R, 77 were NR and 46 were D. A very good association existed between the outcome of the backtests and of the social confrontation tests; the R individuals were mainly the A ones in the social confrontation tests (so-called A/R pigs), while the NR individuals were mainly the NA ones (so-called NA/NR pigs) (Hessing et al., 1993). Furthermore, these different behavioural strategies of A/R and NA/NR pigs correlated highly with coherent behavioural, physiological and endocrine responses to stressful situations and seem to represent two different coping styles (Hessing et al., 1994a). In the present study we selected in each experiment 16 A/R and 16 NA/NR pigs to study individual variation and changes in immune reactivity in reaction to stressful conditions. These stressful conditions were threefold:

1. From all 218 piglets only 150 (experiment 1: n=62; experiment 2: n=88) were observed throughout the experiments. At an age of 30 days these piglets were weaned and relocated to new pens with 10 (experiment 1; 0.77 m^2/pig) or 11 pigs (experiment 2; 0.70 m^2/pig); the first stressor. While most piglets (experiment 1: n=40; experiment 2: n=44) were mixed with non-littermates, some pens consisted of piglets (experiment 1: n=22; experiment 2: n=44) that were not mixed, but kept as a litter together until all pigs were slaughtered at approximately 90 kg live weight (± 21 weeks of age).
2. At an age of 10 weeks all pigs were transported to an adjacent finishing farm and housed in large pens (4.6 m x 6.0 m) with 20 (experiment 1) or 22 (experiment 2) pigs and were mixed partly with familiar and unfamiliar pigs; the second stressor. The farrow-to-finish groups of pigs were also transported, but were of course not mixed and housed in 'standard' finishing pens (3.25 m x 3.25 m) with 10-12 pigs.
3. At and age of 15 weeks pigs housed in the large pens were mixed again; the third stressor.

From the 64 selected subjects of both replicates 24 A/R and 24 NA/NR were distributed ad random over groups of pigs that were mixed and 8 A/R and 8 NA/NR ones over the farrow-to-finish groups. To study individual variation and changes in cell-mediated and humoral immunity in reaction to the three stressful conditions the following in vivo and in vitro immunological tests were performed for all 32 (19♂/13♀) A/R and 32 (15♂/17♀) NA/NR pigs.

In vivo cell-mediated immunity

Phytohaemagglutinin skin test. The phytohaemagglutinin (PHA) skin test was performed as described elsewhere (Blecha et al., 1983). The PHA test was carried out just before weaning (D27 of the experiment), three days after weaning (D33), and furthermore on D40, D47, D52 (Figure 4.1). For each test pigs were injected intradermally in the groin with 0.5 ml of PHA (HA15, Wellcome) (500 μg/ml in sterile physiologic saline) and skin thickness was measured prior to injection and 24 hours later with a spring action cutimeter (Chiron Veterinar Device). Data were expressed in mm increase in skin fold thickness (24 hours post-injection minus pre-injection).

Keyhole limpet haemocyanin skin test. The keyhole limpet haemocyanin (KLH) skin test was performed similar to the PHA skin test. Immediately prior to mixing at 15 weeks all subjects were immunized intramuscularly once with 1 mg of KLH. For immunization, 50 mg of KLH (Fluka) was dissolved in 25 ml sterile 0.9% NaCl and emulsified with 25 ml of the adjuvant Alhydrogel (1.3%, Superfos). Three weeks thereafter (D126 of the experiment; Figure 4.1) pigs were injected intradermally with 0.5 ml of KLH (200 μg/ml in sterile physiologic saline) and the increase (delta)

in skin fold thickness in mm was measured similar to the PHA skin test.

In vitro cell-mediated immunity

Lymphocyte stimulation test. The lymphocyte stimulation test (LST), using PHA and concanavalin A (ConA) as mitogens, was assessed in all selected A/R and NA/NR pigs. The LST was done the day before weaning and the day before mixing at 10 and 15 weeks and subsequently 1 and 2 weeks thereafter (Figure 4.1); so a total of nine assays were performed for each subject. Blood was taken by vene puncture and the blood samples were diluted 1:1 with RPMI 1640 culture medium and layered onto 5 ml of Ficoll-paque (Pharmacia no 17-0840-02) and centrifuged at 400 x g for 30 minutes at room temperature. At the interface of plasma and Ficoll-paque, lymphocytes were collected and washed twice with 0.15 M PBS (pH 7.2). Cells were sedimented by centrifugation at 150 x g for 10 minutes and the pellet was washed twice with RPMI 1640 culture medium. After the final wash lymphocyte concentrations were determined and adjusted to 3 x 10^6 viable cells/ml by adding supplemented RPMI 1640 culture medium containing heat-inactivated fetal calf serum (FCS) (12%). The LST consisted of 100 μl of lymphocytes at 3 x 10^6 cells/ml added in triplicate to wells of a 96-well U-bottom microtitre plate (Nunc). Control cultures were supplemented with RPMI 1640 culture medium without mitogen. Concentrations of 250 μg, 125 μg or 50 μg/ml of PHA or 5 μg, 2.5 μg or 0.5 μg/ml of ConA (Sigma) in the wells were used as mitogenic stimuli. All cultures were incubated at 37°C in a humidified 5% CO_2 - 95% air atmosphere for 48 hours. Lymphocyte blastogenesis was determined by incorporation of ^{3}H-thymidine (0.4 μCi in 20 μl RPMI 1640 culture medium) during the last 18 hours of culture. Cells were harvested for liquid scintillation counting onto filter paper with deionized water by an automated cell harvester. Lymphocyte proliferation of individual animals was calculated as the number of counts/minute (cpm) of each of the mitogen concentration minus the non-stimulated control culture.

Lymphocyte stimulation test with KLH. The LST with KLH as an antigen was similar to the LST using PHA or ConA. The assay was performed three weeks after the subjects had been immunized with KLH (Figure 4.1). Concentrations of 10 μg, 5μg and 2μg/ml of KLH in the wells were used as antigenic stimuli. All cultures were again incubated at 37°C in a humidified 5% CO_2 - 95% air atmosphere but now for 96 hours, while ^{3}H-thymidine (0.4 μCi in 20 μl RPMI culuture medium) was added for the last 18 hours.

Leucocyte-count and -differentiation. Blood samples were taken from all selected pigs on the same days as the LST with PHA or ConA (Figure 4.1). The number of leucocytes were counted by a Coulter counter and expressed in 10^6/ml blood. Blood smears were stained with May-Grunwald-

Giemsa and 100 leucocytes were microscopically counted and differentiated into lymphocytes, neutrophils, monocytes, eosinophils, and basophils, and they were also expressed in 10^6/ml blood.

Humoral immunity

Bovine serum albumin. Immediately prior to the transportation to an adjacent finishing farm at 10 weeks of age all 32 A/R and 32 NA/NR pigs were immunized intramuscularly once in the neck, behind the ears, with 10 mg of bovine serum albumin (BSA), half the dose on each side. For immunization, 500 mg of BSA (Behring) was dissolved in 50 ml sterile 0.9% NaCl and emulsified with an equal volume of the adjuvant Alhydrogel (1.3%, Superfos). Blood was taken 7, 14, 21 and 28 days later (Figure 4.1) and serum was stored at -20°C until required for analysis. Quantification of antibodies specific for BSA was carried out by an enzyme-linked immunosorbent assay (ELISA) procedure. The ELISA procedure used was a modification of those outlined by Engvall and Perlmann (1972). Briefly, polystyrene microtitre plates (Dynatech) were coated with BSA (100 µl/well, 0.5 µg BSA/ml bicarbonate buffer). After incubation (16 h) and washing, 100 µl serum (diluted; 1:3200) was added into each well in triplicate and incubated for 3 h. Thereafter, plates were washed and 100 µl rabbit anti-pig immunoglobulins (IgG/HPR; Dako Patts P164), 1:2000 diluted, was added to each well and incubated for 1 h. Finally, after washing, 100 µl of enzyme substrate (0.004% H_2O_2 (Dako Patts) and 0.4 mg O-phenylenediamine (Sigma) in acetate/citric acid buffer) was added and the colour was allowed to develop in the dark for 10 minutes. The reaction was stopped by the addition of 50 µl 2 mol/L H_2SO_4 and optical densities were read at 490 nm, using an automatic microplate reader (Dynatech). These optical densities were transformed to antibody activity expressed in units/ml (U). For this purpose a standard serum sample, arbitrarily set at 1000 U, was serially diluted from 1:600 to 1:19200. Using the optical densities of this standard serum sample a standard curve was constructed after log transformation and linear curve fitting.

Keyhole limpet haemocyanin (KLH). Blood was taken from all 32 A/R and 32 NA/NR subjects 7, 10, 14, 21 and 28 days after immunization with KLH at 15 weeks of age (Figure 4.1) and serum was stored at -20°C for later analysis. Quantification of specific antibodies to KLH was performed by an ELISA procedure as described above. Briefly, polystyrene microtitre plates were coated with KLH (100 µl/well, 5 µg KLH/ml bicarbonate buffer). Plates were washed twice and 100 µl serum (diluted; 1:300) was added. Optical densities were again read at 490 nm and expressed as units/ml (U). The standard serum sample was serially diluted from 1:30 to 1:7680.

Swine lymphocyte antigen

Swine lymphocyte antigen (SLA) class I haplotypes were serologically determined for all 32 A/R and 32 NA/NR pigs at the laboratory of "Radiobiologie appliquée" in Jouy-en-Josas in France using conventional class I antisera. Heparinized blood samples (20 ml/subject) were diluted with 10 ml of culture medium (containing; RPMI 1640 culture medium; 12% FCS; 10 mM L-glutamine; 4.0 μM 2-mercapto-ethanol; penicillin (100 U/ml) and streptomycin 100 μg/ml), the samples were packed in ice and transported to the laboratory in France were the lymphocytes were prepared and frozen until SLA-typing. A total time of 18 hours elapsed between blood sampling in Denmark and lymphocyte preparation in France. SLA class I haplotypes were defined according to the standard nomenclature as described in Renard et al. (1988).

Statistical analysis

Data were analyzed with the statistical package for social science (SPSS-PC+, version 3.1) (Norusis, 1989). Differences in cell-mediated and humoral immunity were tested by normal analysis of variance (Norusis, 1989). Type of pig (A/R or NA/NR), gender and group (mixed versus non-mixed) were used as factors; the factor gender was later erased from data analysis since it did not significantly contribute to the model. Significant changes in immune reactivity after stressful events were tested with the paired t-test where appropriate, otherwise with special contrast in the repeated measurements of analysis of variance (Norusis, 1989). The effect of mitogen concentration on the lymphocyte proliferation response was tested with the repeated measure of analysis of variance using polynomial contrasts (linear, quadratic and cubic) (Norusis, 1989). The number of pigs with a different SLA-haplotype was analyzed with a Chi^2 (Siegel and Castellan Jr., 1988).

RESULTS

Figure 4.1 represents the design and sampling procedure of the experiments. Analysis showed that results were comparable for both experiments (replicates) and, therefore, they were combined for further analysis.

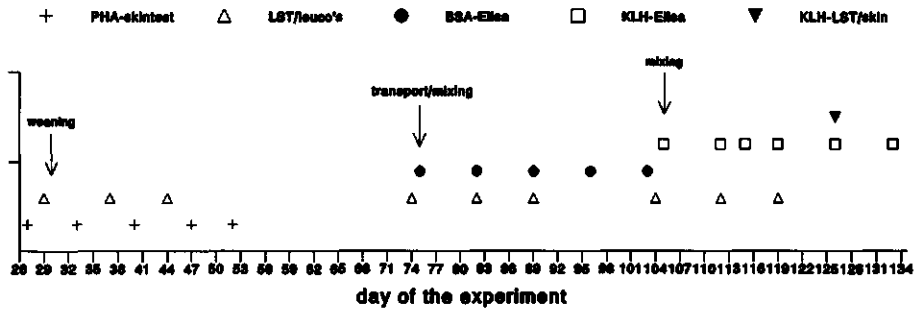

Figure 4.1. Experimental design and sampling procedure. PHA=Phytohaemagglutinin; LST= Lymphocyte stimulation test; leuco's=leucocyte-count and -differentiation; BSA=Bovine serum albumin; KLH=Keyhole limpet haemocyanin.

In vivo cell-mediated immunity

In figure 4.2 data on the in vivo cell-mediated response in the phythohaemagglutinin (PHA) and the keyhole limpet haemocyanin (KLH) skin test are presented. In the PHA skin test both A/R and NA/NR pigs showed a reduced response 3 days after weaning (D33). However, this was only significant for A/R pigs (paired $t_{32} = 2.51$, $P < 0.05$) and not for NA/NR ones (paired $t_{32} = 0.83$, $P > 0.40$). Furthermore, PHA skin test response tended to be higher on D40 and was significantly higher on D47 and D52 in A/R subjects than in NA/NR ones (Figure 4.2). Data analysis showed no significant group (mixed versus non-mixed) effect and no significant group x type of pig (A/R versus NA/NR) interaction ($F < 1$, NS), indicating that the differences in PHA skin test response between both types was similar in each group. KLH skin test response three weeks after mixing at 15 weeks (D126 of the experiment) was also higher in A/R subjects than in NA/NR ones (Figure 4.2), and no significant group effect ($F(1,63)=1.82$, $P > 0.40$) was found.

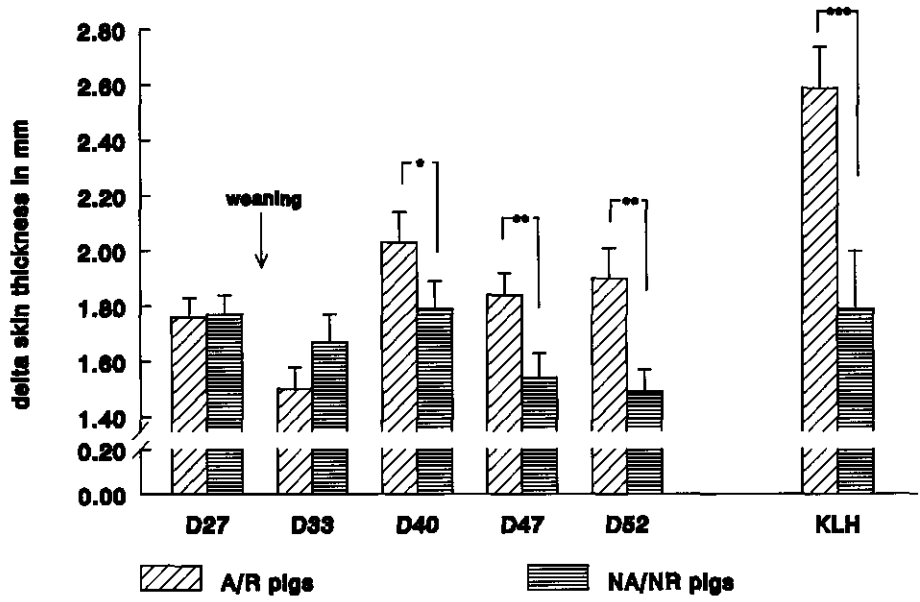

Figure 4.2. Mean (± SEM) skin thickness in mm in the PHA skin test just before weaning (D27) and on several days after weaning, and the KLH skin test performed on D126 of the experiment 21 days after the immunization of all 64 selected A/R and NA/NR subjects. (* = $P < 0.10$; ** = $P < 0.05$; *** = $P < 0.01$).

In vitro cell-mediated immunity

The lymphocyte stimulation test (LST). The LST in experiment 1 failed because massive cell death occurred due to a change in pH of the culture medium used. Therefore, only results are presented from the 16 A/R and 16 NA/NR subjects of experiment 2. The LST showed a significant effect of PHA and ConA mitogen concentration in all nine assays, while polynomial contrasts revealed a significant linear and quadratic effect (data not shown). These results indicate a linear relationship between mitogen activity and mitogen concentration and, in addition, significant quadratic effects resulted from a pronounced increase at the highest concentration of PHA (250 μg/ml) and ConA (5.0 μg/ml). Therefore, data on LST are given of these highest mitogen concentrations (Table 4.1). Control cultures did not differ between A/R and NA/NR pigs (Table 4.1). Although data suggest that A/R pigs had a higher number of counts/minute (cpm) than NA/NR ones, this was significant only on D44 (PHA and ConA) and on D104 (PHA) (Table 4.1). Both types showed a reduced proliferation response the week after weaning and an increase in cpm in the week thereafter (PHA; $F(1,31)=9.49$, $P < 0.01$ and ConA; $F(1,31)=16.16$, $P < 0.01$). The same

holds for the LST after transportation and mixing at 10 weeks of age (PHA; $F(1,31)=2.18$, $P < 0.10$ and ConA; $F(1,31)=29.6$, $P < 0.01$). The reduced response the week after weaning and mixing at 10 weeks was proportionally (in % cpm) always highest for A/R pigs; this was significant ($P < 0.05$) at weaning for PHA (respectively; 26.8% versus 17.4%) and at 10 weeks of age for ConA (respectively; 38.4% versus 23.9%).

Table 4.1. The mean (± SEM) number of counts/minute (cpm) in the lymphocyte stimulation test with PHA (250 μg/ml) and ConA (5.0 μg/ml) - cpm of the control cultures (Δ cpm x 10^{-3}) of 16 A/R and 16 NA/NR pigs on different days of experiment 2.

	PHA		ConA	
	A/R pigs	NA/NR pigs	A/R pigs	NA/NR pigs
D29	23.1 ± 6.6	14.9 ± 4.9	67.5 ± 12.3	62.5 ± 11.6
D37	16.9 ± 4.1	12.3 ± 5.4	52.8 ± 6.5	51.1 ± 8.7
D44	33.9 ± 4.4	26.6 ± 5.5[a]	78.8 ± 6.9	65.4 ± 5.8[a]
D74	32.4 ± 5.2	24.0 ± 6.0	68.5 ± 6.5	56.5 ± 7.2
D82	27.4 ± 4.6	21.1 ± 4.4	42.2 ± 5.4	43.0 ± 6.9
D89	33.0 ± 5.6	27.8 ± 5.9	51.7 ± 6.9	52.8 ± 6.8
D104	37.0 ± 6.9	22.2 ± 4.5[a]	65.1 ± 5.7	56.9 ± 8.3
D112	41.1 ± 5.1	42.3 ± 6.8	75.6 ± 7.3	74.7 ± 8.1
D119	31.5 ± 6.9	37.8 ± 5.7	87.1 ± 7.9	92.4 ± 7.7

[a] Mean cpm differ significantly ($P < 0.05$) from each other.

Mean (± SEM) cpm of the control cultures:
A/R pigs;
D29: 1346 ± 308; **D37**: 1226 ± 140; **D44**: 1831 ± 310; **D74**: 1046 ± 147; **D82**: 828 ± 184; **D89**: 1879 ± 440; **D104**: 986 ± 227; **D112**: 1203 ± 206; **D119**: 1672 ± 555.
NA/NR pigs;
D29: 1199 ± 268; **D37**: 1157 ± 98; **D44**: 2856 ± 812; **D74**: 857 ± 142; **D82**: 816 ± 153; **D89**: 1690 ± 488; **D104**: 725 ± 99; **D112**: 870 ± 141; **D119**: 1753 ± 288

After mixing at 15 weeks mean cpm in the LST did not decrease but surprisingly increased for both types (Table 4.1). The lymphocyte proliferation response of the NA/NR pigs in the mixed groups showed, like the A/R and NA/NR subjects in the non-mixed groups, this significant increase (PHA: paired $t_{12} = 3.24$, $P < 0.05$; ConA: paired $t_{12} = 3.40$, $P < 0.05$) the week (D112) after mixing (Figure 4.3). In contrast, the A/R pigs in the mixed groups showed no significant change in mean cpm (PHA: paired $t_{12} = 0.13$, $P > 0.90$; ConA: paired $t_{12} = 0.18$, $P > 0.90$) (Figure 4.3).

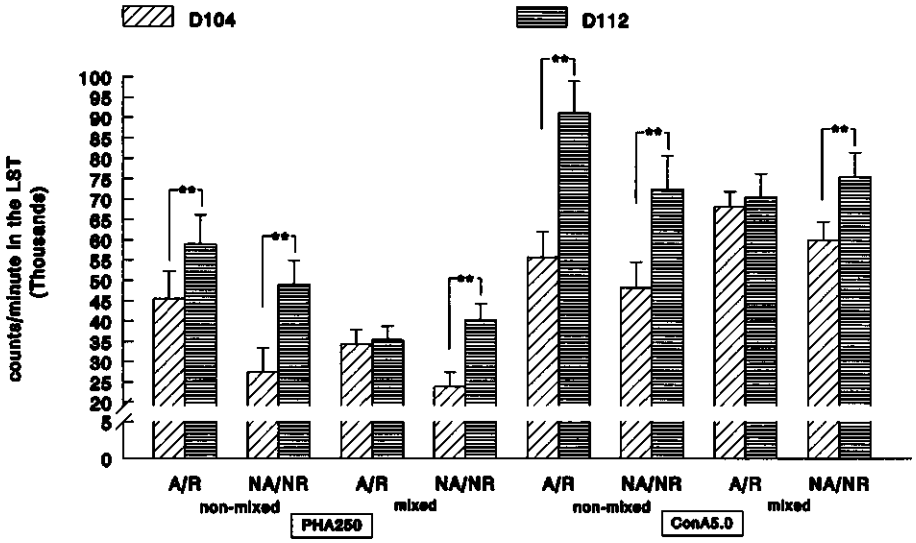

Figure 4.3. Mean (± SEM) counts/minute (cpm) of the lymphocyte stimulation test using PHA (250 μg/ml) and ConA (5.0 μg/ml) of the 4 A/R and the 4 NA/NR in the non-mixed groups and of the 12 A/R and the 12 NA/NR in the mixed groups before (D104) and one week after (D112) mixing at 15 weeks of age in experiment 2. (** = $P < 0.05$)

The LST with KLH three weeks after mixing at 15 weeks demonstrated a significant effect of KLH antigen concentration ($F(3,189)=33.54$, $P < 0.001$). Polynomial contrasts revealed a significant linear ($F(1,63)=111.27$, $P < 0.01$) and cubic effect ($F(1,63)=8.73$, $P < 0.01$). These results again indicate a linear relationship between antigen activity and antigen concentration, while the cubic effect resulted from a pronounced increase at 5 μg/ml KLH (Table 4.2).

Table 4.2. The mean (± SEM) number of counts/minute (cpm) in the LST with KLH in the concentration 2, 5 and 10 μg/ml - cpm of the control cultures of 32 A/R and 32 NA/NR pigs.

KLH	A/R pigs	NA/NR pigs
2 μg/ml	5888 ± 719	4281 ± 619[a]
5 μg/ml	13315 ± 1304	8098 ± 967[b]
10 μg/ml	12769 ± 1613	9167 ± 1057[b]

[a] $P < 0.10$; [b] $P < 0.05$

Mean (± SEM) cpm of the control culture: A/R pigs; 2505 ± 489: NA/NR pigs; 2807 ± 376

In all KLH concentrations the lymphocyte proliferation response was highest in A/R subjects (Table 4.2), while group (mixed versus non-mixed) and interaction type of pig x group were not significantly different.

Leucocyte count and -differentiation

Leucocyte count and -differentiation were done only for the 16 A/R and the 16 NA/NR pigs of experiment 2. The total number of leucocytes, lymphocytes, neutrophils, eosinophils, basophils and monocytes were not significantly different between type of pig (A/R or NA/NR) and between group (mixed versus non-mixed). However, in the weeks after weaning total number of leucocytes and neutrophils increased ($P < 0.01$), while total number of lymphocytes only increased the week after weaning but returned to the initial level the week thereafter ($P < 0.05$). At 10 weeks of age total number of leucocytes ($P < 0.05$) again increased and further increased in the week thereafter. Moreover, the same holds for total number of neutrophils and lymphocytes ($P < 0.10$). After mixing at 15 weeks no significant changes were observed in total number of leucocytes, neutrophils and lymphocytes (data not shown). Furthermore, total number of eosinophils, monocytes and basophils was low and did not change significantly throughout the experiment (data not shown).

Humoral immunity

BSA-Elisa. Although specific BSA IgG antibodies were virtually absent on D7, a pronounced increase occurred at D14, while the highest antibody actvitity was found 21 days after the immunization (Figure 4.4). The overall humoral response to BSA was higher in NA/NR pigs than in A/R ones ($F(1,63)=103.2$, $P < 0.01$), resulting in a higher BSA IgG antibody activity on D14, D21 and tended to be higher on D28 of the former ones (Figure 4.4).

KLH-Elisa. Specific KLH IgG antibodies were already measurable on D7. The overall humoral response to KLH was again highest in NA/NR pigs ($F(1,63)=11.62$, $P < 0.01$), resulting in higher KLH IgG antibody activity (in U) on D7 and D14 and tended to be higher on D21 than in A/R pigs (Figure 4.5).

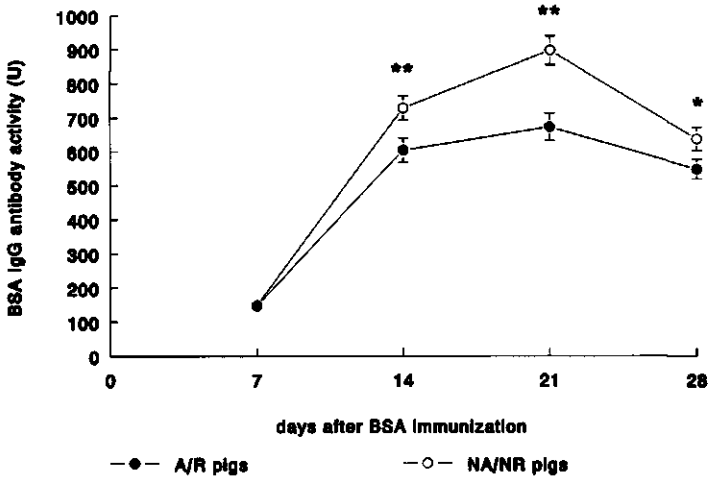

Figure 4.4. Mean (± SEM) BSA IgG antibody activity (in units/ml) for 32 A/R and 32 NA/NR pigs 7, 14, 21 and 28 days after immunization with BSA. (* = $P < 0.10$; ** = $P < 0.05$).

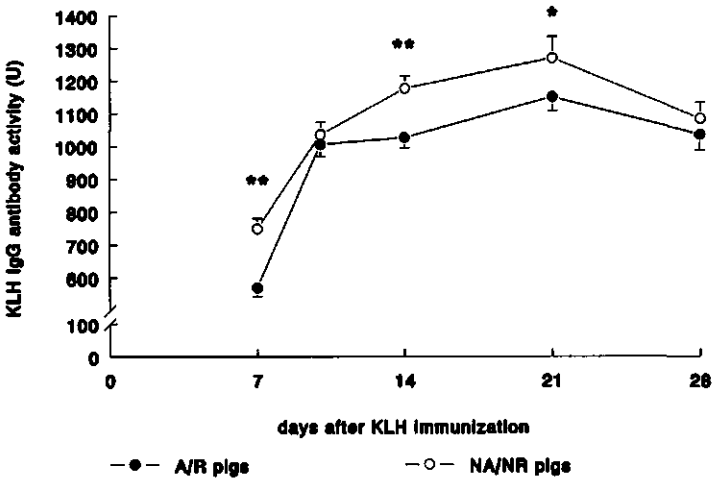

Figure 4.5. Mean (± SEM) KLH IgG antibody activity (in units/ml) for 32 A/R and 32 NA/NR pigs 7, 10, 14, 21 and 28 days after immunization with KLH. (* = $P < 0.10$; ** $P < 0.05$).

Swine lymphocyte antigen

The swine lymphocyte antigen (SLA) class I haplotypes of the 64 selected A/R and NA/NR pigs are presented in table 4.3. A total of 15 different haplotypes were observed, and 5 pigs (=7.8%) were homozygote. SLA-class I haplotype H2, H7 and H10 were present in 43% of

the pigs. The SLA-class I haplotype H10 and H24 received from the boar and H60 received from the sow were more frequent in A/R (respectively; 12.5%, 4.7%, 4.7%) than in NA/NR pigs (respectively; 3.1%, 0%, 0%) (Table 4.3). In contrast, SLA-class I haplotype H15 of the boar was more frequent in NA/NR (12.5%) than in A/R pigs (3.1%) (Table 4.3). All other SLA-haplotypes were equally distributed between A/R and NA/NR subjects (Table 4.3).

Table 4.3. Total number and % of pigs with different SLA-haplotype class I, and furthermore distinguished between the SLA-haplotype received from the boar and the sow.

	Total number of pigs (%)	Boar		Sow	
		A/R	NA/NR	A/R	NA/NR
H1[1]	11 (8.6)	-	-	5 (7.8)	6 (9.4)
H2	18 (14.1)	2 (3.1)	4 (6.3)	6 (9.4)	6 (9.4)
H4	9 (7.0)	1 (1.6)	4 (6.3)	3 (4.7)	1 (1.6)
H6	11 (8.6)	6 (9.4)	3 (4.7)	0 (0.0)	2 (3.1)
H7	19 (14.8)	7 (10.9)	7 (10.9)	2 (3.1)	3 (4.7)
H8	2 (1.6)	-	-	1 (1.6)	1 (1.6)
H10	18 (14.1)	8 (12.5)	2 (3.1)[a]	4 (6.3)	4 (6.3)
H14	7 (5.5)	0 (0.0)	1 (1.6)	2 (3.1)	4 (6.3)
H15	11 (8.6)	2 (3.1)	8 (12.5)[a]	1 (1.6)	0 (0.0)
H24	3 (2.3)	3 (4.7)	0 (0.0)[b]	-	-
H28	1 (0.8)	-	-	1 (1.6)	0 (0.0)
H34	4 (3.1)	-	-	2 (3.1)	2 (3.1)
H48	10 (7.8)	2 (3.1)	3 (4.7)	3 (4.7)	2 (3.1)
H51	1 (0.8)	0 (0.0)	1 (1.6)	-	-
H60	3 (2.3)	-	-	3 (4.7)	0 (0.0)[b]

[1] SLA-haplotypes class I were defined according to standard nomenclature
[a] $\chi^2 = 3.91$; $P < 0.05$; [b] $\chi^2 = 3.07$; $P < 0.10$

DISCUSSION

Over the last decades many studies have shown that stress can alter immune reactivity (reviewed by Griffin, 1989). However, this effect of stress is difficult to value; stress can either suppress, enhance or have no measurable effect on immune reactivity (Blecha et al., 1983; Croiset et al., 1987; Dantzer and Kelley, 1989; Kelley, 1985; Lysle et al., 1990). This is partly due to the magnitude and direction of changes in immunological reactions that depend on the characteristics (i.e., nature, duration, intensity, frequency) of a stressor and its timing in relation to the course of the immune response (Griffin, 1989; Maier and Laudenslager, 1988; Monjan and Collector,

1977). Moreover, the controllability and the perception of a stressor differs between individuals (Dantzer and Mormède, 1983; Wiepkema and Schouten, 1988). In previous experiments we showed that individual differences exist in pigs when coping with conflict situations. Pigs were classified as showing an active (so-called A/R pigs) or a passive behavioural response (so-called NA/NR ones) in social and non-social test situations (Hessing et al., 1993). These two coping styles were associated with consistent differences in physiological activities and baseline endocrine levels in reaction to stress (Hessing et al., 1994a). Recent data demonstrate numerous links between nervous system, neuroendocrine levels and immune processes (Ader et al., 1991) and, therefore, we may assume that the two different coping styles are also reflected in different immune parameters. Indeed, the findings in the present work show that under stress individuals differ in their cell-mediated immunity (CMI) and humoral immunity.

The in vivo T-cell-mediated delayed-type hypersensitivity (DTH) reaction to phytohaemagglutinin (PHA) and keyhole limpet haemocyanin (KLH) differed between A/R and NA/NR ones. Three days after weaning, A/R pigs showed a significant reduced PHA skin test response followed by a rebound effect while thereafter the DTH reaction returned to the initial level. In contrast, NA/NR ones did not show a reduced response directly after weaning but developed a more chronic impairment. These altered DTH reactions are most probably due to hormones associated with stress (Blecha et al., 1983; Westly and Kelley, 1984). One of these hormones is glucocorticoid; T-cells involved in the DTH reaction are most sensible for its suppressive effects (Westly and Kelley, 1984). This suggests that A/R pigs may have a more reactive glucocorticoid response in the first phase of stress than NA/NR ones. Although, in previous experiments (Hessing et al., 1993b) we were unable to substantiate this, several other studies have shown that behavioural active individuals indeed demonstrated a more reactive glucocorticoid response under stress (Bohus et al., 1987; Hanssen and Damgaard, 1993). However, our previous experiments (Hessing et al., 1994a) did show that NA/NR pigs had higher basal levels of cortisol compared to A/R ones; this may explain the chronic impairment of the DTH reaction in the former ones. The skin test using KLH as a specific antigen again showed a lower response in NA/NR than in A/R ones indicating that the differences in CMI between subjects remained consistent over time.

The in vitro lymphocyte stimulation test (LST) using concanavaline A (ConA), PHA and KLH suggested that A/R subjects had again a higher CMI than NA/NR ones. However, shortly after stress the proliferative response in the LST using PHA and ConA was reduced more in A/R than in NA/NR pigs. This suggests that A/R pigs experience stressors used in the present work (i.e., weaning, new environment, transportation, mixing) as more stressing than NA/NR ones did. Studies in rodents (Benus et al., 1987; 1990) and in pigs (Hessing et al., 1994a; Schouten and

Wiepkema, 1991) showed that behavioural active individuals may have more problems to adapt to changes in their environment than the behavioural passive individuals that perform better under changing conditions. The LST after mixing at 15 weeks using PHA and ConA differed with the LST around weaning and at 10 weeks in that proliferative responses surprisingly enhanced. Immunoenhancement or adaptation of immune responses after stress has been described elsewhere (Lysle et al., 1990; Kelley et al., 1982), especially when stressors were mild (Croiset et al., 1987) or after chronic exposure to the same stressor (Irwan and Livnat, 1987; Monjan and Collector, 1977), and this may account for the effects seen in the present study. Nonetheless, this immunomodulation was not observed in A/R pigs a week after mixing, this may again suggest that they experience such stressors as more stressing. Although this may explain the different immune reactivity of A/R versus NA/NR pigs in the mixed groups, it does not elucidate the immunoenhancement observed in both subjects of the non-mixed groups.

The changes in total number of leucocytes and leucocyte subsets show that the stressors applied in the present study have long lasting effects. Lymphocytosis and neutrophilia after stress have also been described elsewhere, while others found opposite results (reviewed by Westermann and Pabst, 1990). Important to realize is that the number of leucocytes and leucocyte subsets in the peripheral blood represent only a very small portion (only 2%) of the total pool of these cells found in the body (Westermann and Pabst, 1990). Moreover, the mechanisms underlying the changes in the number of these cells are still unclear. The most plausible explanation is that changes in subsets are due to a shift of the cells from the peripheral blood to the marginal pool or vice versa (Butcher, 1986; Westermann and Pabst, 1990). It has been suggested that the neuroendocrine stress response accounts for those effect (Crary et al., 1983; Westly and Kelley, 1984). Therefore, we have to be careful when relating stress simply with changes in number of leucocytes and leucocyte subsets in the peripheral blood system.

Stress is known to have an effect on humoral immunity as well; while antibody production is often reduced by acute stress, it can be enhanced by chronic stress (Griffin, 1989). In our experiments levels of specific antibodies to bovine serum albumin (BSA) and KLH were highest in NA/NR pigs. This may reflect a different stress susceptibility of A/R and NA/NR individuals. However, it is striking that low CMI is accompanied by high humoral immunity in NA/NR pigs but opposite (high CMI and low humoral immunity) in A/R pigs. Such contradictory responses between the humoral- and the cell-mediated immune system have been reported by others (Mason, 1991). According to Mason (1991) the balance between these two immune mechanisms can be shifted by glucocorticoids. Glucocorticoids may enhance antibody synthesis by increasing interleukin (IL)-4 a B-cell growth factor that enhances expression of the major histocompatibility

complex (MHC) class II molecules on the surface of B-cells, but also suppresses CMI by reducing IL-2 production and T-cell proliferation (Ashman, 1989; Smith, 1989). We observed high basal levels of cortisol in NA/NR pigs (Hessing et al., 1994a), so the above described explanation may hold for the effect seen in the present study. Yet, other neuroendocrine substances (i.e., ß-endorphin, somatotropin, catecholamine, thyroid hormone and others) that vary under stress may also cause differential effects on immune reactivity (Blalock, 1989).

A central role in the immune reactivity and disease resistance is played by cell surface glycoproteins encoded by the MHC to distinguish self from non-self cells (Lamont, 1991; Tonegawa, 1993; Vaiman, 1987). In our study we found 15 different serologically defined class I swine lymphocyte antigens (SLA), this is not much different described in other reports (Renard et al., 1988; Rothschild et al., 1986). In our commercially bred pig population the number of homozygote pigs (7.8%) was relatively high, but even somewhat lower compared to other pig populations (Gautschi and Gaillard, 1990; Rothschild et al., 1986). Moreover, some defined SLA-class I haplotypes in this study (H10,H15,H48) were present in much higher frequencies that those found in foreign pig populations (Gautschi and Gaillard, 1990; Rothschild et al., 1986), while SLA-class I haplotypes frequently present in these foreign population (H5,H9,H12) were totally absent in ours. These phenomena may be explained by the breed-specific nature of SLA class I haplotype distribution (Renard et al., 1988). Not all defined SLA class I haplotypes in this study were equally distributed between A/R and NA/NR individuals. Although, it remains unclear if these observed differences have any clinical relevance to different immune functioning or disease resistance.

In conclusion, data of the present study demonstrate in pigs the existence of consistent individual differences in immune reactivity. Active A/R pigs have a higher in vivo and in vitro CMI to nonspecific and specific antigens, while after stress CMI was more reduced in A/R than in NA/NR ones. In contrast, humoral immunity was highest in NA/NR pigs. These findings show that measurement of immune reactivity in vivo as well in vitro is an important tool to define how animals cope with environmental demands. Above all, these findings emphasize that attention for the individual organism, in this, is crucial.

ACKNOWLEDGEMENTS

The personnel of the pig farm, under supervision of Anders Eklundh Larsen, of the National Institute of Animal Science, Research centre Foulum, Denmark is thanked for excellent care of the pigs and Jan van Beek and Rie Krukow for extensive practical work. Anna Halborg Madsen of the laboratory of the Research centre Foulum performed very accurately the LST and the BSA- and KLH-Elisa. Mariëtte Prince made perfect blood smears and the laboratory of the Animal Health Service in South Netherlands performed the leucocyte differentiation. Piet Wiepkema, Willem Schouten and Henk Parmentier of the Agricultural University of Wageningen in the Netherlands made valuable comments on this manuscript. Mike Nieuwland of the Agricultural University, section Immunology kindly provided information about the KLH tests. Noell Bourgeaux of the laboratory of Radiobiologie appliquée in Jouy-en-Josas in France is greatfully acknowledged for her excellent and quick analysis of the SLA-class I haplotypes.

Chapter 5

PRACTICAL IMPLICATIONS OF INDIVIDUAL BEHAVIOURAL CHARACTERISTICS IN PIGS

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Accepted for publication in: Livestock Production Science (in press)

PRACTICAL IMPLICATIONS OF INDIVIDUAL BEHAVIOURAL CHARACTERISTICS IN PIGS

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ABSTRACT

At a commercial closed farm we investigated how far it profits to compose groups of pigs based on individual behavioural characteristics. In a non-social backtest each of 206 piglets tested were individually restrained in a supine position and classified as resistant (R; > two escape attempts), intermediate (I; two escape attempts) or non-resistant (NR; < two escape attempts). Based on the outcome of two successive backtests in week 1 and 2, piglets were classified as R (n=86), NR (n=94) or doubtful (D; n=26). At nine weeks of age the remaining pigs were grouped into six pens with only R pigs, six pens with only NR pigs and six pens with both R and NR ones (10 pigs/pen). Two pens with only D pigs were excluded from the experiment. Agonistic behaviour recorded just after mixing was significantly higher in R pens compared to NR pens and to R/NR pens. The average daily weight gain in grams/day was higher of the pigs in the R/NR pens compared to the pigs in the other pens. Furthermore, carcass weight and meat% was somewhat higher and carcass classification was better of the pigs in R/NR pens compared to the pigs in the other pens. This resulted in a higher payment of approximately 10 Dutch Florins per pig of the R/NR pens. Post mortem examination showed that less pigs in the R/NR pens had pleurisy than pigs in the R pens and in the NR pens. The number of pigs with stomach wall damage was highest for pigs in the NR pens. The present study suggests that better group composition of pigs in intensive husbandry can be realized when based on the individual behavioural characteristics of a pig. This is profitable for the animal and their farmer.

Keywords; Pigs; Individual behavioural characteristics; Productivity; Costs

INTRODUCTION

The way vertebrates react when their homeostasis is threatened largely determines their survival (Moberg, 1985). Biological reactions to relevant changes in their internal or external milieu consist of a chain of behavioural, physiological and neuroendocrinological activities regulated by the brain (Ader et al., 1991). However, individuals of the same species may value or experience a threatening situation differently. Therefore, the same stressor can have differential effects on separate individuals (Dantzer and Mormède, 1983; Henry and Stephens-Larson, 1985). Existent evidence suggests that basically two different ways of dealing with conflict situations exist, an active or a passive one; each individual seems to be predisposed to one or the other

coping strategy (Benus et al., 1987; 1990; Schouten and Wiepkema, 1991; Wiepkema and Schouten, 1988). This preference, determined by genetic constitution and early life experiences (Suomi, 1987; Van Oortmerssen et al., 1985) is consistent over time and over situations (Benus et al., 1987, 1990) and is visible in different behavioural, physiological and neuroendocrine programs (Bohus et al., 1987).

Previously, we found such differences among individual pigs. These differences were already detectable at an age of 1-2 weeks, were consistent over time and over situations, and were also associated with different physiological, endocrine and immunological responses to stressful situations (Hessing et al., 1993, 1994a, b). The existence of these individual coping styles may be important in understanding the complex social relations among pigs living in social groups. Such pigs may disturb or support each other depending on their individual characteristics and this may have great practical value. In intensive husbandry pigs are housed in prolonged confinement and in high density but, above all, they are constrained in their choices of penmates. Some types of individuals may simply not fit each other and high levels of social stress can be expected. A group composition based on individual behavioural characteristics of pigs may stabilize the social structure of the group much quicker and better. A stable social structure will reduce stress and will be beneficial for the individual pig and the farmer. The present study investigates in how far group composition, based on individual coping characteristics, may benefit the growing up of fattening pigs.

MATERIAL AND METHODS

Housing and animals

The experiment was performed in two farrowing units at a commercial closed farm in the Netherlands. In each unit ten sows (Dutch Landrace x Great Yorkshire) farrowed within five days. A total of 213 piglets was born from these 20 sows. Each farrowing pen (5.2m^2) had a heated concrete lying area and metal slats. Male piglets were castrated three days after birth. At weaning (day 28) the sows were removed; piglets were not mixed. At nine weeks of age all pigs were transported to two adjacent finishing units with ten pens each (7m^2) and all pigs were mixed. Pigs were fed *ad libitum* from day 10 until transportation to the finishing units with a normal commercial diet (energy content; 13.9 MJ ME/kg feed; 19.6% crude protein; 3.2% crude fat; 1.02% total lysin). While two different diets were used during the finishing period; a grower feed was fed to the pigs from 25 to 40 kg live weight (energy content; 14.2 MJ ME/kg feed; 17.9%

crude protein; 3.8% crude fat; 1.0% total lysin) and a finishing feed was fed to the pigs from 40 to 107 kg live weight (energy content; 14.7 MJ ME/kg feed; 16.7% crude protein; 5.8% crude fat; 0.88% total lysin). Water was supplied *ad libitum* using a water-nipple.

Backtest

All piglets, (n=206; seven piglets died in the first week), were tested twice during the suckling period, once in the first week and once in the second week. The procedure of the backtest has been described in detail elsewhere (Hessing et al., 1993). Latency to first resistance and the number of vocalizations was noted down during both backtests. The classification of each individual piglet was based on the outcome of the two backtests. To be classified as a R individual it should have shown twice a R outcome or once a R and once an I outcome. Comparable rules were used for the classification of a NR individual. All remaining piglets were classified as doubtful (D). From the 206 piglets tested during the suckling period, 197 pigs were fattened.

Mixing procedure

At nine weeks of age these 197 pigs were transported to two adjacent finishing units (10 pens/unit) at the same commercial farm. Pigs were grouped in pens (10 pigs/pen) with only R pigs (R pens; n=6), with only NR pigs (NR pens; n=6), or with both R and NR pigs (R/NR pens; n=6). Three of the R/NR pens consisted of 4 R and 6 NR pigs, two R/NR pens consisted of 5 R and 5 NR pigs and one R/NR pen consisted of 4 R, 5 NR and 1 D pig. The remaining D pigs (n=17) were grouped in two pens: one with 10 D pigs and one pen with 7 D pigs and three unknown pigs from another unit. These two pens were excluded from data analysis. During the finishing period three pigs ('retailers') of the 180 (=1.7%) were removed from the finishing units. Until the end of the finishing period (23-25 weeks of age) pigs were not mixed nor relocated.

Agonistic behaviour

Immediately after the relocation and mixing procedure two observers recorded for one hour the occurrences of agonistic behaviour (Jensen, 1982; McGlone, 1985) among pigs of the R, NR and R/NR pens; most agonistic behaviour occurs in this period (Symoens and Van den Brande, 1969). In one-minute sampling observations the number of pigs showing agonistic behaviour was recorded for all pens; 60 samples per pen per hour were obtained. These samples were summed and expressed as the total agonistic behavioural score per pen.

Daily gain

Pigs were individually weighed just before the relocation and mixing procedure, and their average daily weight gain (ADWG; in grams/day) for the growing-finishing period was calculated as: (estimated live weight at slaughterhouse - live weight at start of growing period) x 1000 / number of days of the growing period. Cold slaughter weight was converted into an estimated live weight at slaughter using the following formula from SIVA (1991): estimated live weight at slaughter (in kg) = [cold slaughter weight x 1.3 + (0.0025 x (83 - cold slaughter weight))].

Post mortem examination

Pigs were slaughtered at approximately 107 kg live weight (23-25 weeks of age). Each pig got a ham tattoo number before transportation to the slaughterhouse. In combination with the ear tattoo number the carcasses could be identified. The carcasses were classified into AA, A, B or C according to E.C. classification standards and meat percentage of the individual carcasses was determined by the Hennessy Grading Probe. The veterinary staff and meat inspectors of the Meat Inspection Service at the slaughterhouse recorded the following pathological lesions as described in detail by Elbers (1991): pneumonia, pleurisy and abscess(es) in the lungs; pericarditis; inflammation of the leg; arthritis; inflammation of the tail; skin lesions and partially affected or condemned liver. Furthermore, heart and stomach of each individual pig were removed and examined at the Animal Health Service in the Southern Netherlands. The heart was checked for congenital anomalies and the occurrence of subepicardial and subendocardial lesions, inflammations, scar tissue and haemorrhages. Stomach wall damage was scored as described in detail elsewhere (Hessing et al., 1992). Briefly, stomach wall damage was observed at the pars oesophagus area and expressed in six codes based on the severity of the damage. These codes were respectively, code 0 (=normal pars oesophagus); code 1 (=light hyperkeratosis: less than 50% of the surface); code 2 (=severe hyperkeratosis: more than 50% of the surface); code 3 (=hyperkeratosis and small erosions: less than 5 and shorter than 2.5 cm); code 4 (=hyperkeratosis and more and bigger erosions: 5 or more and/or longer than 2.5 cm); code 5 (=hyperkeratosis and many and big erosions: more than 10 and/or longer than 5 cm, and/or ulcer with or without bleeding or occlusion (=stenose) of the entrance of the oesophagus into the stomach).

Statistical analysis

Data presented were analyzed using the Statistical Package for Social Science (SPSS-PC+ version 3.1) (Norusis, 1989). Differences in behaviour in the two backtests were tested by normal analysis of variance. Differences in total agonistic behaviour score and the average daily weight

gain among pens were analyzed using Tukey's multiple range test. The number of pigs with or without pathological alterations was tested with the Cross tabulation method, which measures a Chi-square. The average daily weight gains of pigs with or without pathological alterations were again analyzed by normal analysis of variance (Norusis, 1989).

RESULTS

Backtest

In the backtests 206 piglets were tested. The number of R, NR and D individuals was respectively 86 (43♂/43♀), 94 (49♂/45♀) and 26 (12♂/14♀); obviously 180 of the 206 pigs (=87.4%) could be classified as a R or NR individual after two backtests. Gender was equally distributed among the groups. Moreover, no differences were found in the distribution of R, NR and D piglets among litters (data not shown). Latency to first resistance and number of vocalizations in the two backtests (BT1 and BT2) are presented in table 5.1.

Table 5.1. Mean (± SEM) latency (sec.) to first resistance and number of vocalizations in two backtests (BT1 and BT2) of R, NR and D piglets.

Commercial Farm		R piglets (n=86)	NR piglets (n=94)	D piglets (n=26)
BT1;	Latency	18.1 ± 1.3[a]	38.7 ± 1.9[b]	27.4 ± 2.7[c]
	Vocalizations	41.0 ± 2.0[a]	20.0 ± 1.9[b]	29.4 ± 3.2[b]
BT2;	Latency	17.0 ± 1.1[a]	40.1 ± 1.6[b]	30.8 ± 2.9[c]
	Vocalizations	50.7 ± 2.1[a]	25.6 ± 1.9[b]	38.2 ± 3.5[c]

[a,b,c] Mean with different superscript differ significantly from each other; $P<0.05$

In BT1 and BT2 latency to first resistance was lower and number of vocalizations was higher for R piglets compared to NR ones. The D piglets showed an intermediate latency to first resistance and number of vocalizations compared to R and NR individuals (Table 5.1). From the 206 piglets tested during the suckling period 197 pigs (86 R, 93 NR and 18 D) were fattened.

Agonistic behaviour

Initially, after relocation and mixing pigs explored their new environment, but agonistic interactions started within minutes. The occurrences of agonistic behaviour among pigs differed

among pens ($F(2,16)=14.31$, $P < 0.001$). Post hoc analysis showed that the occurrences of agonistic behaviour was significantly higher ($P < 0.01$) in pens with only R pigs (66.9 ± 12.9) compared to the pens with only NR pigs (28.3 ± 11.6) and to pens with R and NR pigs (36.7 ± 14.7) (Figure 5.1).

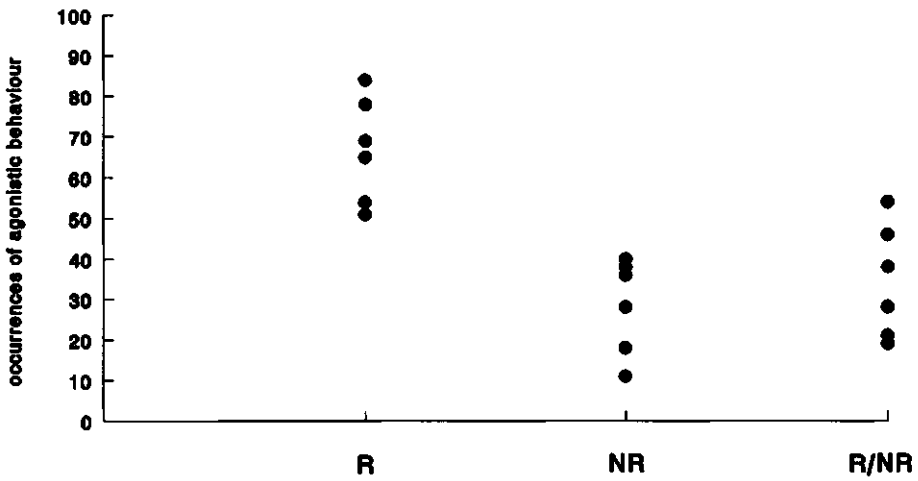

Figure 5.1. The occurrences of agonistic behaviour in one hour after mixing per pen with only R pigs (R pens), only NR pigs (NR pens) or with R and NR pigs (R/NR pens).

Daily gain

The average daily weight gains (ADWG) during the fattening period are given in figure 5.2. This ADWG in grams/day was higher in R/NR pens (801 ± 18) than in NR pens (773 ± 26) or in R pens (761 ± 34). Post hoc analysis showed that this difference in ADWG was significant ($P < 0.05$) between R/NR and R pens; the difference in ADWG between R/NR and NR pens was not significant ($P = 0.11$). Furthermore, the coefficient of variation (=CV) of ADWG was lower among R/NR pens (CV=2.2%) than among NR pens (CV=3.4%) or among R pens (CV=4.4%) (Figure 5.2). The same holds for the average CV of the ADWG **within** each pen; R/NR pens: CV=7.1% (range 6.2% to 9.0%); NR pens: CV=10.5% (range 7.4% to 14.4%) and R pens: CV=11.8% (range 7.3% to 16.9%); this shows that the variation in ADWG between and within R/NR pens was lowest and highest for R pens.

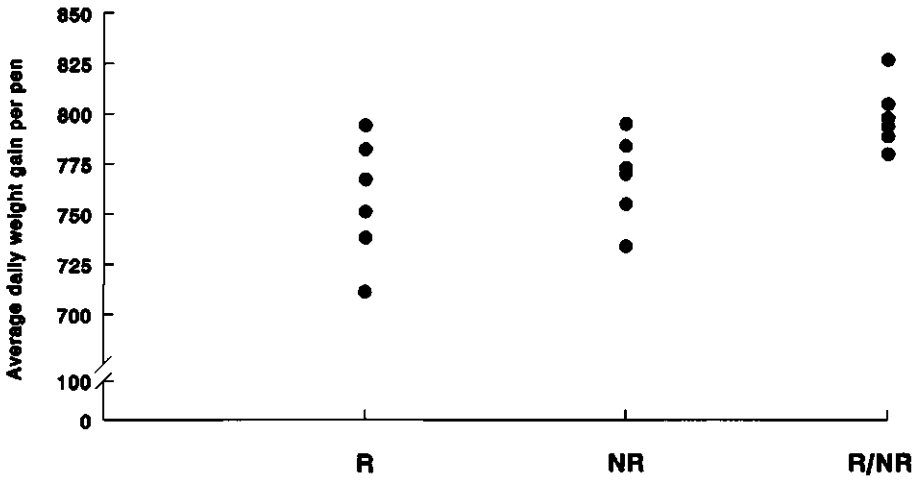

Figure 5.2. Average daily weight gain (grams/day) of the fattening period for R, NR and R/NR pens.

Post mortem examination

Carcass weight, carcass classification and meat% of the pigs are presented in table 5.2.

Table 5.2. The carcass weight in kg, the number of pigs with carcass classification AA, A, B or C and meat% of the pigs grouped in R, NR or R/NR pens.

	R pens (n=6; 57 pigs[1])	NR pens (n=6; 57 pigs[1])	R/NR pens (n=6; 58 pigs[1])
Carcass weight (kg)	88.9 ± 3.3[a]	89.5 ± 2.2[a]	91.2 ± 2.0[b]
Meat%	54.9 ± 2.9[x,y]	54.7 ± 3.3[x]	55.6 ± 3.0[y]
Classification[2]; AA	10	8	14
A	40	43	41
B	7	6	3
C	0	0	0

[1] five pigs were missed, so data from 172 of the 177 (=97.2%) pigs brought to the slaughterhouse were available.
[2] Carcass classification was better of the pigs in the R/NR pens ($\chi^2 = 6.21$, $P < 0.05$) compared to the pigs in the other pens.
[a,b] Mean with different superscript differ significantly from each other; $P < 0.05$.
[x,y] Mean with different superscript tends to differ from each other; $P < 0.10$.

The higher carcass weight, better carcass classification and higher meat% of the pigs housed in the R/NR pens (Table 5.2) resulted in a higher payment of approximately 10 Dutch Florins (1 US $ ≈ 1.85 Dutch Florin) per pig (calculation; see Appendix). The post mortem examination in the slaughterhouse revealed that the prevalence of pneumonia (4/172 pigs = 2.3%), pericarditis (3/172 pigs = 1.7%), inflammation of the leg (2/172 pigs = 1.2%) and partially affected liver (3/172 pigs = 1.7%) was low. Pleurisy, however, did occur in 36 of the 172 pigs (=20.9%) and there was a tendency (χ^2 = 4.63, df=2, P < 0.10) that its prevalence was lower among pigs in the R/NR pens (7/58 pigs = 12.1%) than among pigs in R pens (16/57 pigs = 28.1%) or NR pens (13/57 pigs = 22.8%). Pigs with pleurisy had a significant (P < 0.05) lower ADWG of 18 grams/day than pigs without this lesion. The post mortem examination at the Animal Health Service in the Southern Netherlands showed that 38 of the 172 pigs (=22.1%) had severe haemorrhages on the heart muscle. This prevalence did not differ significantly among pigs of different pens (R pens: 16/57 pigs = 28.1%; NR pens: 10/57 pigs = 17.5% and R/NR pens: 12/58 pigs = 20.7%). Pigs with this heart alteration did not have a significant lower ADWG (- 10 grams/day, P > 0.10) than pigs without this alteration. The number of pigs (in %) with stomach wall damage per code is presented in figure 5.3.

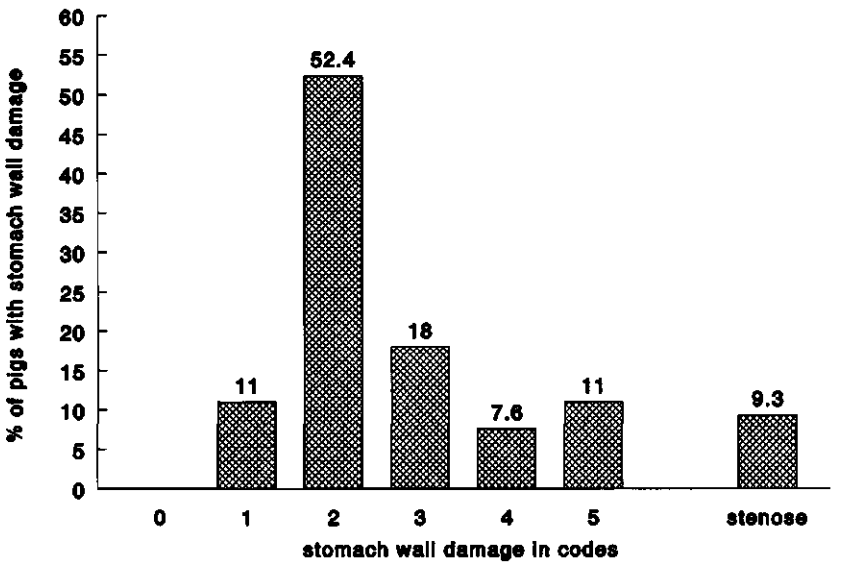

Figure 5.3. Percentage of pigs with stomach damage per code (as described in material and methods). Stenose is also included in code 5.

Figure 5.3 shows that 63.4% of the pigs had a light stomach wall damage (code 1 and 2), whereas 36.6% had a severe damage (≥ code 3). Stenose was present in 9.3% of the pigs. Besides that, data showed that the prevalence of severe (≥ code 3) stomach wall damage was higher (χ^2 = 6.41, df=2, P < 0.05) among pigs in NR pens (29/57 pigs = 50.9%) than among pigs in R pens (19/57 pigs = 33.3%) or among pigs in R/NR pens (17/58 pigs = 29.3%). Pigs with severe stomach wall damage had a significant (P < 0.01) lower ADWG of 35 grams/day compared to the pigs without a severe stomach wall damage. Pigs with a stenose had even a 65 grams/day lower ADWG (P < 0.01) than pigs without a stenose.

DISCUSSION

In the intensive pig husbandry social aspects in housing and management of the animals are often neglected. Pigs are confined into small barren environments, in high density or sometimes housed individually. Furthermore, social groups are frequently disrupted and unfamiliar pigs are housed together; this despite the many studies (Blecha et al., 1985; Fleshner et al., 1989; Mormède, 1988) showing the negative effects of such procedures. Probably, most stressing for pigs is that they are constrained in their choices for penmates. Previously we showed that individual pigs behaved differently in conflict situations. Like in other species (Benus et al., 1987; 1990; Corson and Corson, 1976; Slater, 1981; Von Holst, 1986), one type reacted actively, whereas the other type reacted passively when stressed (Hessing et al., 1993). The different types may differently influence the social organization of a group when unfamiliar pigs are housed together. This may strongly influence social stability. If so, a good group composition of animals is a function of the individual characteristics of each group member.

In the present study we composed groups of pigs based on their individual behavioural characteristics. The distinction among pigs was based on two backtests performed in the first two weeks of life and under practical farm conditions. The results showed that after two backtests 87.4% of the pigs could be classified as a resistant (R) or as a non-resistant (NR) individual. Behaviour shown by the R and the NR individuals was in full agreement with previous findings (Hessing et al., 1993), suggesting that the outcome of the backtests has a general significance.

As expected agonistic behaviour directly after mixing at nine weeks was highest in pens with only R pigs and lowest in pens with only NR ones. The same was found in a previous experiment (Hessing et al., 1993). This suggests two general but individual behavioural coping strategies in pigs; the behaviour pattern of the resistant and aggressive pig reflects an active

behavioural response, whereas the non-resistant and non-aggressive one reveals a passive coping style.

The differences in the average daily weight gain (ADWG) over the entire finishing period of 14-16 weeks show that the group composition of pigs had great effects. Pigs in the R/NR pens had a 40 grams/day higher ADWG than the pigs in the R pens and 28 grams/day higher than the pigs in the NR pens. Besides that, a low variation was found in ADWG among the R/NR pens and also within each R/NR pen compared to the other pens. Due to a low CV in ADWG, finishing pens and finishing units can be emptied quicker and in this way the all in-all out system can be better maintained. This outcome provides the possibility of an appropriate cleaning and disinfection procedure to prevent diseases and to lower the prevalence of pathological lesions (Elbers, 1991).

The pigs' health is a matter of balance between environmental influences and the disease resistance of the animal (Moberg, 1985). Disease problems and/or pathological lesions will appear when this balance is disturbed by adverse management and climatic conditions (Scheepens et al., 1991; Tielen, 1974). The lesions observed in our study were not equally distributed among pigs of the different pens. For instance, the prevalence of pleurisy was lower among pigs in the R/NR pens than among pigs in the R pens or NR pens. Because management and climatic conditions were equal for all pens the differences observed are most likely due to a different disease susceptibility of the pigs in different pens. Disease susceptibility of an animal can be increased by social stress (Ebbesen et al., 1991; Gross, 1984) and this suggests that social stress was lower among pigs in the R/NR pens than among pigs in the other pens. This lower social stress may be caused by a stronger social stability in the R/NR pens than in R and NR pens. Furthermore, the present study illustrates the high prevalence of severe stomach wall damage (36.6%) in pigs in present day intensive husbandry. Similar findings have been reported by others (Fleerakkers et al., 1992). Severe stomach wall damage was higher among pigs in the NR pens than among pigs of the R pens or R/NR pens. It might be that passive 'copers' (NR pigs) react to stress conditions (i.e., social instability in the NR pens) with an increased parasympathetic nervous activity (Bohus et al., 1987; Hessing et al., 1994a), which may specifically influence the stomach wall. In contrast, active 'copers' (R pigs) react predominantly with a high sympathetic nervous reactivity affecting heart and bloodvessels (Hessing et al., 1994a). Although the prevalence of heart alterations was indeed highest among the pigs in the R pens the differences were not significant.

Both pleurisy and stomach wall damage explain partially the lower growth performance of the pigs in the R pens and in the NR pens. Pleurisy accounted for a growth retardation of 18 grams/day per pig, which agrees with other reports (Lundeheim, 1988; Tielen et al., 1978). A

severe stomach wall damage (≥ code 3) caused a growth retardation of 35 grams/day and this increased to 65 grams/day in cases of a stenose (= code 5). Therefore, disturbance of the well-being of the animals have great economical implications. Moreover, pigs of the R/NR pens had a somewhat higher carcass weight and meat% and a better carcass classification, which resulted in an essential higher payment of approximately 10 Dfl. per pig. Further research is needed to substantiate this.

The present findings clearly show that it is worthwhile to compose groups of pigs based on their individual behavioural characteristics. Groups consisting of both R (active 'copers') and NR pigs (passive 'copers') may better fit each other resulting in higher productivity and fewer pathological lesions than groups with only active or with only passive 'copers' together. It is not really surprising that putting active 'copers' together (R pens) may not be a good procedure, because among such animals aggressive behaviour will escalate. Totally unexpected, however, was the relatively low performance level of the pure NR groups. Low performance of a group may reflect a relatively low social stability, this implies that social stability is best in mixed R/NR groups. This leads to the surprising conclusion that under stressful conditions R and NR animals may need each other in order to quickly develop a stable social organization. This intriguing biological aspect of social organization of animals appears to be an entirely novel one and needs further research.

The present study demonstrates that social aspects in intensive pig husbandry are very important. Good management implies not only perfect climatic conditions and feeding, but also attention for and understanding of the social environment of the farm animals.

ACKNOWLEDGEMENTS

Family Jenniskens is greatly acknowledged for their hospitality and the opportunity they gave us to carry out the experiment on their farm in Hunsel (Limburg) in the Southern Netherlands. We thank Paula Hamm for her extensive practical work. The personnel of the slaughterhouse of Coveco in Weert in the Southern Netherlands kindly cooperated with our requests concerning the slaughtering procedure of our pigs. Carl van Doorn, student of the Agricultural University Wageningen and Ger Verhaegh, Ruud Leenen and Jelle Bakker of the Animal Health Service in the Southern Netherlands helped in gathering the heart and stomach from each individual pig.

Appendix:

Calculation of the payment for the slaughter pigs

Standard average payment (in 1992) per kg (classification A and 54.0% meat):		3.011 Dfl.
Carcass classification:	AA	+ 0.10 Dfl.
	B	- 0.05 Dfl.
Meat%:	+ 1.0%	+ 0.04 Dfl.
	- 1.0%	- 0.05 Dfl.

1 US $ ≈ 1.85 Dutch Florins (Dfl.)

	R pens (n=6; 57 pigs[1])	NR pens (n=6; 57 pigs[1])	R/NR pens (n=6; 58 pigs[1])
Carcass weight (kg)	88.9 ± 3.3	89.5 ± 2.2	91.2 ± 2.0
Meat%	+ 0.9% (= + 0.036 Dfl.)	+ 0.7% (= + 0.028 Dfl.)	+ 1.6% (= + 0.064 Dfl.)
Classification; AA	10 (+ 0.10 Dfl.)	8	14
A	40 (-)	43	41
B	7 (- 0.05 Dfl.)	6	3
C	0	0	0
Average payment/pig	271.89 Dfl.	272.78 Dfl.	282.41 Dfl.

[1] five pigs were missed, so data from 172 of the 177 (=97.2%) pigs brought to the slaughterhouse were available.

In conclusion,

Pigs housed in the R/NR pens at the commercial farm had a higher payment of approximately 10 Dfl. per pig compared to pigs housed in the R pens or in the NR pens

GENERAL DISCUSSION

GENERAL DISCUSSION

There is a growing interest in studies on behavioural variability among individuals within a population. While in the past this variation has been viewed primarily as a statistical problem, nowadays we become aware of its significance in providing information on strategies or roles in social behaviour, on personality traits, and individual recognition.

Individual behavioural differences have been reported within many species like humans (Glass, 1977), monkeys (Sapolsky, 1990; Suomi, 1987), dogs (Corson and Corson, 1976), crickets (Cade, 1981), ruffs (Van Rhijn, 1973), zebra finch (Slater, 1974), calves (Wiepkema et al., 1987), goats (Lyons et al., 1989), sagebrush lizards (Martins, 1991), minnow shoals (Murphy and Pitcher, 1991), beech marten (Hansen and Damgaard, 1993), tree shrews (Von Holst, 1986), mice and rats (Benus et al., 1987; 1990; Fokkema et al., 1988); thus behavioural variation among individuals seems a universal phenomenon.

Even despite the diversity in species some similarities in this behavioural variability are present. First, behavioural differences among individuals become overt especially when stressed (e.g., novelty, intensive housing), or when competing over territories, a sexual partner, social position, feed, and so forth. While some individuals take the initiative others do not or just the opposite; this suggests a dichotomy in behaviour among individuals within a population. Hence, it is postulated that such a dichotomy may reflect a rather general but individual way of handling stressful situations (i.e., 'coping'). In this context, Henry and Stephens (1977) previously presented a model that also specifies the relationship between 'coping' and physiological functioning. When the organism is challenged two possible responses (fight-flight or conservation-withdrawal) may emerge each with its own characteristic biological pattern (Henry and Stephens, 1977). The reactions of the so-called active coper is characterized by an increased blood pressure, a high reactivity of catecholamines and corticosteroids and performance of offensive or escape behaviour. In contrast, the so-called passive coper is characterized by a decreased blood pressure, a low reactivity of catecholamines and corticosteroids and performance of freezing behaviour (Benus et al., 1987; 1990; Bohus et al., 1987). Furthermore, each coping style may be successful in being adapted to different environmental conditions (Benus, 1987; 1990). A surprising but intriguing point is that each individual appears to be predisposed to one or the other coping strategy. This strongly suggests a genetic and ontogenetic basis, while recent life experiences may play an important role also (Suomi, 1987; Van Oortmerssen et al., 1985).

Based on the results of the present study three major topics will be discussed in more detail. First, do behavioural differences in pigs represent idiosyncratic response patterns? The

second topic deals with the way the individual behavioural characteristics in pigs relate to different autonomic nervous and immune reactivity under stress conditions. Finally, the third topic views the relevance of applying behavioural studies in pigs to the practical situation of intensive husbandry.

INDIVIDUAL BEHAVIOURAL CHARACTERISTICS

Like other species, farm animals express great individual behavioural variability in adapting to environmental stressors. Most studies emphasized the individual differences in performing environmentally induced disturbed behaviour like sham-dustbathing in hens (Liere et al., 1991), stereotypies of tethered sows (Schouten et al., 1991) and young pigs (Dybkjaer, 1992), sham-sucking on the prepuce of a penmate in group-housed veal calves (De Wilt, 1985), and stereotypies in individually-housed veal calves (Wiepkema et al., 1987). Recently it has been suggested that these differences may represent individual coping characteristics of the animals involved (Schouten and Wiepkema, 1991), although some scepticism remains (Dantzer, 1991). Nonetheless, it demonstrates the different individuals' appraisal in farm animals of stressful conditions in intensive husbandry.

The present study provides further evidence of behavioural differences among individual pigs living under farm conditions. These differences were already detectable at an early age and remained highly consistent over time (chapter 2 and 5). Moreover, a strong consistency existed in the individual response patterns between social (aggressive behaviour in social confrontation tests and after mixing; chapter 2) and non-social contexts (resistance behaviour in backtests and behaviour to novelty; chapter 2 and 3). The aggressive individuals in the social confrontation tests at an early age were again highly aggressive after mixing later on, were resistant in the backtests, and had a tendency to escape a novel environment; moreover they explored rapidly and superficially a novel object in their environment. The non-aggressive individuals, however, were again non-aggressive after mixing, hardly tried to escape a novel environment, and explored the novel object gradually but more intensively. In short, the pigs show consistent individual behavioural strategies when coping. Their individual idiosyncratic characteristics implicate a bimodal distribution in coping behaviour, active (aggressive/resistant) vs. passive (non-aggressive/non-resistant) and this resembles the two behavioural stress responses (fight-flight vs. conservation-withdrawal) as presented by Henry and Stephens (1977). However, does this tendency towards a bimodal distribution imply that a normal distribution of behavioural parameters within a population never

exists? Furthermore, does it mean that an individual will never change its predisposed behavioural coping strategy? Does a passive 'coping' individual never show an active behavioural response or vice versa? Surely these assumptions are too simplistic! First, a bimodal shape distribution of behaviour occurs within a population only when the individuals are challenged. Thus a normal distribution of behaviour may predominante in situations were challenges are absent and this implies that the different types of individuals become only visible when seriously stressed. The present study does not indicate that the individual pigs may change their predisposed coping style, nevertheless this a possibility. For example, studies among male house mice showed that some passive individuals adopted an active behavioural strategy but only when the stressor (active shock avoidance) was easy to control (Benus et al., 1990). Although not emphasized in their study, active male house mice did perform freezing behaviour as a passive response to a resident male house mouse, but significantly less than passive ones did (respectively; 45% against 60% of the observation time) (Benus et al., 1987). In the present study, the non-aggressive pigs did show aggressive behaviour after mixing and, could even win fights, but they did so much less than the aggressive ones (chapter 2). Moreover, in contrast with the aggressive pigs the non-aggressive ones hardly ever initiated fights or chased an already defeated conspecific (Table 2.2, chapter 2). Therefore, it is important to realize that the two different types of animals do not possess a completely different set of behaviours, but they differ primarily in their degree of behavioural responsiveness to stress.

Summarizing, the individual pigs show time-consistent patterns of behavioural responses to social and non-social challenges; this illustrates idiosyncratic characteristics in pigs. Besides that, the different characteristics in pigs suggest a bimodal distribution in behavioural coping responses when stressed. They are active or passive copers.

INDIVIDUAL PHYSIOLOGICAL CHARACTERISTICS

Vertebrates cope with a relevant environmental challenge not only behaviourally, but also physiologically. This latter activity involves integrated pathways like the autonomic nervous system, the neuroendocrine system and the immune system (Ader et al., 1991). Behavioural and physiological responses are highly integrated and, therefore, both aspects must be measured simultaneously to fully comprehend individual variation to stress (Moberg, 1985). In other words, a multidisciplinary approach in stress research is essential. Because the individual pigs differ in their degree of behavioural responsiveness to stress (cf. chapter 2), as a rule different physiologi-

cal responsiveness may be expected per individual.

Hence, in the present study the individual behavioural characteristics in pigs have been related to different physiological strategies, and the major findings are summarized in table 6.1.

Table 6.1. Major differences in baseline level and reactivity of the autonomic nervous system, the endocrine system and the immune system and differences in pathological state of the active and the passive pigs in response to several stressors applied in the present study.

TARGET	STRESSOR	ACTIVE PIGS	PASSIVE PIGS
Autonomic nervous system			
Heart rate reactivity	restraint	⇑⇑⇑	⇑
Heart rate reactivity	novelty	⇑⇑⇑	⇔/⇓⇓
Endocrine system			
Cortisol baseline	-	LOW	HIGH
Cortisol reactivity	novelty	#	#
Cortisol reactivity	ACTH	⇑⇑⇑	⇑⇑⇑
Immune system			
Cell-mediated immunity;			
Baseline	-	HIGH	LOW
First phase of stress	housing/management	⇓⇓⇓	⇓
Chronic phase of stress	housing/management	⇔	⇓⇓⇓
Humoral immunity;	housing/management	⇑	⇑⇑⇑
Pathological alterations			
Heart muscle deviations	-	⇑⇑⇑	⇑
Stomach wall damage	-	⇑	⇑⇑⇑
Adrenal weight	-	⇔	⇑⇑⇑
Surface area adrenal cortex/medulla	-	⇔	⇔

Baseline levels in HIGH or LOW and reactivity in: ⇑=increase; ⇓=decrease; ⇔=no change; number of arrows arbitrarily indicates degree of change; #=not clear.

This summary unambiguously shows that the individual behavioural characteristics are associated with the individual physiological characteristics (cf. chapter 3 and 4), and that each behavioural strategy has its own characteristic physiological pattern as argued by others (Bohus et al., 1987; Henry and Stephens, 1977; Von Holst, 1986). Subsequently, different patterns of the autonomic nervous system and the immune system between active and passive pigs will be further outlined.

Autonomic Nervous System

The autonomic nervous system (ANS) consists of two main divisions; the sympathetic (SNS) and the parasympathetic (PNS) one, and both are important in monitoring the body's

physiology. The two divisions of the ANS function mainly antagonistically in that the effects on the organs they innervate oppose each other. For example, enhanced SNS activity to stress will result in increased cardiovascular reactivity (increase in blood pressure and heart rate), whereas enhanced PNS activity leads to the opposite (decrease in blood pressure and heart rate). This autonomic balance on the cardiovascular system has been extensively studied in stress research, especially in humans (Glass, 1977). Studies in humans, but also in other species (Fokkema et al., 1988; Schouten et al., 1991; Von Holst, 1986) showed that individual differences in cardiovascular reactivity depend largely on the characteristics of the individual behavioural responses to cope with environmental challenges. The present study substantiates these findings (cf. chapter 3).

For instance, the pigs that resist strongly to the restraint procedure of the backtests have a higher mean heart rate (HR) than the pigs that hardly resist (cf. chapter 3). More intriguing is the marked difference between the active (A/R) and the passive (NA/NR) pigs in their cardiac response to novelty (i.e., the falling bucket) in the (second) open field test. While HR of the active pigs clearly increases (tachycardia), HR of one-third of the passive pigs even decreases (bradycardia) (Figure 3.4, chapter 3). In general, tachycardia is associated with enhanced SNS-adrenal medulla activity, while bradycardia suggests enhanced vagal (i.e., PNS) activation. This implies that the cardiac autonomic balance may shift within the individual pigs when challenged. Whereas the active pigs have a predominance of the SNS over the PNS, the passive ones have a more dominant parasympathetic drive; these findings correspond with other reports (Bohus et al., 1987; Fokkema et al., 1988).

The question arises why individuals have a predominance for either SNS or PNS when challenged? Of course this should be related to the main function of SNS and PNS; SNS controls the blood circulation in a state of emergency (fight-flight), when blood is mainly distributed to the active muscles (cardiac and skeletal), whereas PNS acts to conserve body's resources and restores homeostasis. However, whether active or passive pigs have respectively increased/decreased plasma catecholamines, or increased/decreased density, and/or sensitivity of cardiac ß-adrenergic receptors, or decreased/increased sensitivity of acethylcholine to account for the effects reported are yet unclear.

Normally, the ANS reactivity to stress is a physiological adaptive response, but when stress reactions are prolonged or occur very often, overstimulation of the ANS can eventually become harmful for the organism. Overstimulation of the SNS response to stress may lead to cardiovascular disorders (e.g., hypertension and atherosclerosis), while overstimulation of the PNS may cause problems for the digestive tract e.g., stomach ulcerations. If so, one may expect to find heart deviations in the active pigs, but more stomach wall damages in the passive ones. Indeed,

the present findings substantiate this, in that the more active pigs have subepicardial and subendocardial haemorrhages on the heart muscle (cf. chapter 3), whereas in contrast the more passive pigs have stomach wall damages when these latter pigs were grouped together (cf. chapter 5). Additionally, previous experiments showed that more pigs that were submitted to mixing procedures (used in practice) had heart alterations (subepicardial lesions) as compared to the pigs that were not mixed; however, mixing did not lead to more pigs with stomach wall damages. A striking finding was that the pigs with a subepicardial lesion did not have stomach wall damages, whereas the pigs with stomach wall damages did not have a subepicardial lesion (Hessing et al., 1994, in preperation).

In conclusion, active and passive pigs display a shift in their ANS balance when stressed. Active pigs have a dominant SNS response, whereas passive individuals have a more dominant PNS response, but each response having its own price.

The Immune System

Nowadays, the immune system is no longer viewed as a physiological system that operates independently, but as an adaptive mechanism that closely integrates with other homeostatic processes within an organism (Ader et al., 1991). The function of the immune system is to discriminate between self and non-self, and mount responses to sustain health and resistance to potentially life threatening non-self elements e.g., bacteria and viruses. The immune response is affected not only by genetic and environmental factors, but also by non-immunological stressors (reviewed by Griffin, 1989). Especially these latter stressors have drawn much attention in recent animal studies. The influence of stress on the immune system is well accepted. However, the direction and magnitude of the immune responses and the effects of stress on immunity show great variations between and within species (Griffin, 1989, Kelley, 1985). The immunological effects may vary not only with the characteristics and chronicity of the stressor, but also with the degree of predictability and control available to the individual animal. Therefore, the immune reactivity and subsequently the disease resistance will differ in reaction to different kinds of stressors, but also among individuals to the same stressor. Certainly this latter variation raises the intriguing question: "why are some individuals more susceptible to a certain stressor than others?"

This intriguing question also holds for the results described in the present study. For instance, some pigs die or some show severe clinical signs in response to an Aujeszky virus, while others remain clinically healthy (cf. chapter 1). Part of this variation in immune reactivity and

disease susceptibility is associated with the individual social status; subordinate pigs are more at risk than subdominant and dominant ones. However, even some dominant pigs die or express severe clinical signs indicating that also other factors next to social status have a role in this. In fact, the present work underlines that the coping style during stress of the individual pig is an important factor determining individual variation in immune reactivity. Active pigs (i.e., A/R) display a higher in vivo and in vitro cell-mediated immunity (CMI) to nonspecific (PHA and ConA) and specific (KLH and BSA) antigens than the passive (i.e., NA/NR) ones (cf. chapter 4). In reaction to the stressors applied in the present study (i.e., weaning, new environment, transportation, mixing) the active pigs have a reduced but temporary CMI response in the first phase of stress, while the passive pigs show a more delayed and a more chronic impairment. These differences can be due to hormones associated with stress (Kelley, 1985). The most likely candidate for this is glucocorticoid that normally has suppressive effects on immune responses (Westly and Kelley, 1984). This implies that the active individuals may have a more reactive glucocorticoid response in the first phase of stress as has been reported by others (Bohus et al., 1987; Hansen and Damgaard, 1993), however, this remains unclear in the present study (Table 6.1). The consistent higher basal levels of cortisol present in the passive pigs may eventually lead to a chronic impairment of the CMI as seen in these individuals. Completely unexpected is the higher humoral immunity (KLH and BSA antibody activity) in the passive pigs compared to the active ones. This suggests a converse relationship in the individual pig between cell-mediated and antibody-mediated immune responses to the same stressor, and even to the same antigen used. An interesting question remains whether such a contrary response, low CMI with high humoral immunity and vice versa, is mere coincidence or a coherent entity? Several recent findings favour the latter idea because also in other species (humans: Weiss, 1993; mice: Mason, 1991; rats: Mormède et al., 1988; hens: H. Parmentier, personal communication) such a converse relationship in immune responses has been shown. A possible interpretation for this converse relationship may be the switch in the expression of the different subsets of T-helper cells during CMI responses.

Recent data in humans (Weiss, 1993) and mice (Mosmann and Coffman, 1987) have shown that the T-helper cells can be divided into two types: T_H1 and T_H2. Both T_H-cells secrete interleukin 3 (IL-3) and granulocyte/macrophage-colony stimulating factor (GM-CSF). However, T_H1-cells make IL-2 and gamma-interferon (IFN-γ) leading to inflammatory responses, but not B-cell stimulatory factor (BSF-1), whereas T_H2-cells secrete BSF-1 (i.e., IL-4), but not IL-2 and IFN-γ. Thus, CMI as expressed by inflammation is more dependent on T_H1-cells and humoral immune activity on T_H2-cells. Interestingly, these two types of T_H-cells impede each other, and as

a rule any extrinsic signal that promotes one of the two will inhibit the other, and the balance between the two types may change (Weiss, 1993). Thus, it is of great interest to find out whether different subsets of T_H-cells also exist in pigs or that T_H-cells secrete different lymphokines in response to different signals.

A switch in T_H-cells can be induced by a neuroendocrine signal and, again glucocorticoid is a likely candidate, because it promotes BSF-1 (i.e., IL-4) but suppresses IL-2 (Mason, 1991). In other words glucocorticoid may stimulate antibody production, but may suppress inflammation. The differences in basal cortisol levels among the individual pigs of the present study (Table 6.1) seem to substantiate this explanation. However, many other neuroendocrine substances (e.g., ACTH, ß-endorphin, met-enkephalin, VIP, GH, etcetera) may influence immune responses since receptors for these substances (including glucocorticoid) have been identified on the immune cells (Blalock, 1989; Jankovic, 1989).

Besides many neuroendocrine also neural signals influence the immune system (Ader et al., 1991). This concept of a neural-immune communication is supported by the presence of autonomic nerve fibres (mainly sympathetic-noradrenergic ones) in primary (bone marrow and thymus), and secondary (spleen, lymph nodes and gut-associated) lymphoid organs (Jankovic, 1989). Furthermore, innervation is possible through the presence of α- and ß-adrenergic receptors, and of different subtypes of cholinergic receptors on the immune cells (Felten and Felten, 1988). This communication channel is bidirectional, because the immune system signals the central nervous system and, the ANS through lymphokines and neuroendocrine (e.g., ACTH, ß-endorphin) like substances, which influence the neural activity (Blalock, 1989). The main goal of this integrated communication circuitry is to withstand any extrinsic and intrinsic signals that may threaten the homeostasis of each organism. As described previously, the active and the passive pigs display a shift in their ANS activity when stressed; respectively a more dominant SNS or PNS activity. This difference in neural activity in the individual pigs may have a profound influence on the immune reactivity that may result in different immune responses as seen in the present study. But most studies (Felten and Felten, 1988) have emphasized the SNS activity on immune responses and only little is known about the possible effects of PNS on immunity.

In conclusion, active and passive pigs clearly differ in their immune responses to conflict situations. Especially the converse relationship between the two types of T-cell mediated immunity (i.e., inflammation or antibody responses) within an individual pig is striking and needs further research. In general, this shows that in stress research the immune reactivity should be focused on multi-immune parameters, because the immune system is a network of interactions providing various routes for counteracting non-self.

PRACTICAL IMPLICATIONS OF BEHAVIOURAL STUDIES

Over the last 30 years pig husbandry has changed from an extensive into an intensive production system, which resulted in impressive growth in animal productivity. However, due to this process of expansion and intensification pigs' environmental and housing conditions have greatly altered, causing considerable behaviour and health problems. The high incidence of these problems associated with an increasing use of veterinary drugs (Elbers, 1991), high mortality and culling rates, and high prevalence of pathological lesions in pigs (Elbers, 1991), and in sows (Geudeke, 1991) at slaughter, clearly show that we have reached a point of intensive farming beyond the pigs' ability to adapt. Are there no alternative more appropriate pig production systems? Indeed, some efforts are being made e.g., more extensively housed slaughter pigs, outdoor pig production and so on. But alternative housing systems will only succeed when they can compete economically against present-day intensive systems. Besides politics, also consumers have a main role in this process. Fortunately, there is growing public interest in animal welfare, and this will stimulate production systems and management procedures more suited to the animals involved. Nonetheless, quality of intensive pig production systems can be much improved and behavioural observations are an indispensable tool for this.

'Abnormal' behaviours in pigs are undoubtedly related to the intensive housing systems. Many of these 'abnormal' behaviours are in fact components of normal behavioural patterns directed to inappropriate environmental stimuli. For example, tail biting can be due to many factors, but crucial is the impossibility for the pigs to practise normal foraging and rooting behaviour (Ruiterkamp, 1985). Therefore, I prefer to subscribe these behaviours as disturbed and not as abnormal. Basically, disturbed behaviour represents a pig's attempt to adjust to an 'abnormal' environment, although this often leads to injuries to themselves or to penmates. Hence, disturbed behaviours in pigs represent clear signals for environmental and/or management deficiencies in housing, climatic, feeding, or social conditions. These deficiencies may also have detrimental effects on performance and health. This suggests a close relationship between behaviour, performance, and disease as illustrated in figure 6.1.

In practice, low productivity or even clinical diseases in the pigs are viewed and treated as the main indicators of environmental and/or management deficiencies. In this context, disturbed behaviours have often been neglected although they sometimes even may precede and directly impair performance and disease resistance. This implies that we should address the underlying causes of behavioural problems in pigs, and not treat them symptomatically (e.g., tail docking, teeth clipping, keeping the pigs in the dark, tranquilizers, etcetera). Behavioural observations are

essential in this respect, but under practical conditions far from easy. For instance, when entering a unit pigs become agitated and, consequently they must be observed either from outside the unit or after allowing them to habituate to the human presence. Furthermore, the average animal hardly exist (cf. chapter 2) and the same holds for the average farm. Behavioural problems in pigs must be viewed within the environmental and the management contexts of the farm in question. Subsequently, to detect and correct behavioural problems a good biological knowledge about the pigs' environmental and behavioural needs is essential. Unfortunately, this knowledge is lacking not only in farmers, but also often in others (veterinarians, zootechnicians, nutritionists) who frequently visit the pig farms. Therefore, it is highly recommended to first enhance the basic knowledge of ethological principles before analysis and treatment of behavioural problems in practice can be undertaken successfully.

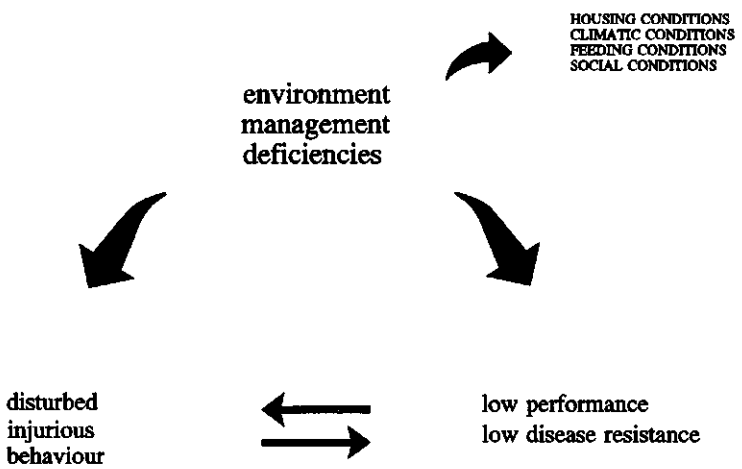

Figure 6.1. The close relationship between environmental and management deficiencies and disturbed behaviour and low performance and disease resistance in pigs.

In conclusion, without doubt behavioural observations in practice deliver essential indicators of deficiencies in the pig production systems, although some pieces of the behavioural puzzle are still missing. For example, why are disturbed behaviours in the pigs a major problem in one farm but only a minor problem in another comparable one? Why have some farms, despite the presence of behavioural problems in the pigs, a higher animal productivity compared to other

farms lacking these problems? Furthermore, why are certain treatments of behavioural problems in the pigs successful on one farm but unsuccessful on another? No doubt, these are difficult but important issues to be solved by the applied ethologists.

Social conditions

In intensive husbandry, the social environment of the pigs has been underestimated and neglected. Domesticated pigs living under farm conditions are, like the pigs living in the wild, highly social animals, and their group structure is based upon a dominance hierarchy. The social relations within a group are established through agonistic interactions among group members. These interactions already start during the very first weeks of their life, although at that time the social confrontations are usually playful. The eventual social stability in the group will reduce stress, which is beneficial for each individual pig. However, in practice the maintenance of such a stable social group causes much stress due to adverse housing conditions and management procedures. Pigs are housed in a barren environment, and often in high density groups from which it is impossible to escape. In addition, stable social groups of pigs are disrupted several times through management procedures (e.g., weaning and start of the finishing period). It is common knowledge that when unfamiliar pigs meet they severely fight to establish a new social hierarchy. These fights can cause injuries like ear, flank, and leg wounds, and subsequently impair performance and increase the risk for diseases e.g., inflammations. As expected, the best management procedure is not to mix the pigs, but keep them as a group together (farrow-to-finish) as has been suggested in the so-called Stress-Specific-Free (SSF) system (Scheepens et al., 1992). However, this is as yet for most of the intensive housing systems not feasible. At presence, groups of pigs are composed by their farmers merely upon uniformity. Presumably, such a homogenous and highly artificial group composition is not the best choice for each individual pig. Some animals may simply not fit each other and subsequently threaten the social stability in the group leading to high levels of social stress.

The present findings (cf. chapter 2) show consistent individual differences in the pigs when coping with stressful situations. These individual coping strategies can have important implications in understanding the social relations among group-housed pigs. A proper group composition of the pigs is a function of the individual behavioural characteristics of each group member. In fact, the distribution of the different types of pigs in a stable social structure may not be arbitrary. If so, knowledge on this aspect can be useful for the farmer to compose groups of

pigs on better grounds than is the case now. The present study demonstrates that there are better grounds. For this one first has to determine the coping style of each individual pig preferable at an early age, and in an easy and quick way, and with reliable outcomes. The backtest (cf. chapter 2 and 5) meets these requirements and can be carried out during the suckling period in which the piglets are already handled and restrained several times by the farmer. This method has been tested at a commercial closed farm (cf. chapter 5). After two backtests, 87.4% of the 1-2-week-old piglets could be classified as an resistant (R) or an non-resistant (NR) individual. At the start of the finishing period (nine weeks of age) the pigs were mixed and grouped accordingly to these individual behavioural characteristics. Pens were filled with only R pigs (R pens), with only NR pigs (NR pens) or with R and NR pigs (R/NR pens) together. The high occurrence of agonistic behaviour immediately after mixing in the R pens, but low occurrence in NR pens again indicates the presence of two individual coping characteristics (cf. chapter 2), active (aggressive and resistant; A/R pigs) or passive (non-aggressive and non-resistant; NA/NR pigs). In general, the higher average daily weight gain (ADWG), lower coefficient of variation of ADWG, lower prevalence of pathological lesions at slaughter, and somewhat higher carcass quality of the pigs in the R/NR pens as compared to the R pens and the NR pens strongly suggests that groups consisting of both R (active 'coper') and NR pigs (passive 'coper') better integrate. It may be postulated that the performance of a group of pigs is a reflection of the social stability of that group. If so, this implies that the social stability is best in the R/NR pens resulting in higher productivity and fewer pathological alterations than in the R pens or in the NR pens. As expected, only active 'copers' together (R pens) is not a proper group composition because aggressive behaviour will escalate among such pigs. Totally unexpected, is that only passive 'copers' together (NR pens) is not a proper group composition either. Obviously under adverse conditions active and passive pigs need each other to quickly develop a stable social organization. This intriguing and novel biological aspect of social organization of pigs needs some further comments.

As described previously, the individual behavioural characteristics of the pigs (active or passive) will become visible only when seriously challenged. Therefore, putting only active or only passive pigs together will not result in many problems under non-stress ad libitum conditions. But why do they depend on each other in stressfull situations? In practice, conditions are far from easy; the pigs are confined into small barren environments, and housed in high densities, and as a rule space for each individual is limited. In addition, they have to compete over food, water, lying place, social contacts, and so on. These limited farm conditions may cause strong social friction in the group. The presence of passive individuals may be of great advantage for the entire social group, since they may gradually withdraw from the active indviduals giving the latter more

effective space; this may have a quieting effect. Thus, the passive pigs seem to 'control' the behavioural response of the active ones, and this may reduce the social tension in the group without causing severe problems for the former. In contrast, the passive individuals may benefit from the active ones in that the latter will take the initiative under limited conditions, whereupon the former will follow shortly after. This indicates that animals living in social groups, even under intensive farm conditions, observe and depend on each other to cope with environmental demands.

In conclusion, the complementary individual behavioural characteristics of the active and the passive pigs under stress will result in a better socially integrated group. Subsequently, a well socially integrated group will be more successful in solving their problems in a predictable and controllable way. This is beneficial for each individual group member and the group as a whole will perform better. Of course this is still theory and it would, therefore, be of great interest to further study this new biological element in social organization of animals.

SUMMARY

SUMMARY

Introduction

The main aspect of this thesis is individual behavioural variation. Behavioural variability among individuals within a population may provide information on strategies or roles in social behaviour, on personality traits and individual recognition. Generally, this behavioural variability becomes overt in stressful situations. Recent data have shown the existence of basically two different coping strategies, active or passive. These different coping styles resemble the two (classical) behavioural stress responses, fight-flight vs. conservation-withdrawal, each with its own characteristic biological pattern. The success of the individual coping response depends on the environmental conditions and, therefore, it is highly surprising that each individual appears to be prediposed to one or the other coping strategy. This suggests a genetic or ontogenetic basis, but recent life experiences will have a significant role also. The idiosyncratic response pattern to a challenge has been shown in many species (humans; monkeys; dogs; tree shrews; etcetera), and hence it may be postulated that this also holds for pigs. If so, these individual behavioural characteristics will have important practical implications in understanding the social relations among group-housed pigs in intensive farm conditions. A stable social structure in the group, and thus a proper group composition, may be a function of the individual behavioural characteristics of each group member. However, until now little research has been conducted to reveal possible patterns underlying a proper group composition in pigs, and subsequently how such mechanisms could be applied in intensive pig husbandry. The present study aims at these aspects.

Social status

In chapter 1, the individual variation in disease susceptibility and immune reactivity of pigs is described in relation to their individual social status in a stable social group. This social status was determined by the outcome of social ranking fights and food competition tests. There was a substantial agreement between the social status determined by these ranking fights and food competition tests. Since these tests were made at quite different ages (respectively; during the suckling period, and on day 50, on day 65, and on day 100), this indicates a relatively stable social structure in the group. At an age of approximately ten weeks, all pigs were challenged intranasally with an Aujeszky virus. Mortality and morbidity were highest among subordinate pigs compared to subdominant and dominant ones. A specific lymphocyte stimulation test, using purified Aujeszky virus as an antigenic stimulus, showed that the cell-mediated immunity (CMI) against the Aujeszky virus was higher for the dominant pigs than for the subdominant and

subordinate ones.

These findings showed that there were large individual differences in immune reactivity and disease susceptibility in pigs partly related to their individual social status in the group. However, social behaviour of an animal that lives in a social organization is also determined by its individual way of handling stressful situations i.e., its coping strategy. Therefore, the individual coping response may well be another basis for different internal biological programs, which may eventually lead to individual differences in disease susceptibility. In chapter 2 the hypothesis was tested whether consistent individual behavioural characteristics in pigs exist.

Individual behavioural characteristics

During the suckling period, piglets were classified as aggressive or as non-aggressive individuals in two successive social confrontation (SC) tests by two observers. Substantial agreement in this classification existed between observers and between both SC tests. Moreover, the aggressive behavioural elements observed after mixing at 10 and again at 15 weeks of age were mainly shown by pigs that were classified as the aggressive ones in the two social confrontation tests shortly after birth; this indicates that the behavioural response pattern of the individuals remained consistent over a long period of time. In a non-social backtest piglets were restrained in a supine position for sixty seconds, and classified as resistant (R; > two escape attempts), intermediate (I; = two escape attempts), or as non-resistant (NR; < two escape attempts). Based upon the outcome of five successive backtests piglets were eventually classified as R (n=95), as NR (n=77), or as Doubtful (n=46). Results showed that two backtests performed on piglets at an early age may suffice for practical use. A striking finding was the good association that existed between the outcome of the backtests and of the SC test. The individuals that resisted in the backtests were the aggressive ones in a social situation, while the non-resistant individuals were the non-aggressive ones. This association and the strong consistency over time strongly suggests an individual behavioural strategy to cope with conflict situations. The idiosyncratic characteristics indicate a bimodal distribution in coping behaviour in pigs; they are active (aggressive and resistant; A/R) or passive (non-aggressive and non-resistant; NA/NR) pigs.

Individual physiological characteristics

The way these individual behavioural strategies in pigs relate to different behavioural, physiological, and endocrine responses under stress conditions is illustrated in chapter 3. For this, 32 A/R and 32 NA/NR pigs were selected and individually tested in an open field (OF) test at three and eight weeks of age. While A/R pigs more than NA/NR ones tried to escape the OF, the

A/R pigs vocalized less during the OF procedure than the NA/NR ones did. Furthermore, the A/R ones explored a novel object inside the OF rapidly and superficially, whereas the NA/NR ones did so gradually but more intensively. The cortisol response to the OF (t=0/t=90) differed between the A/R and the NA/NR pigs. The cortisol response to a farmacological dosis $ACTH_{1-39}$ (2.5 IU/kg live weight/pig) at three and eight weeks of age showed no significant differences between both types of pigs. Nonetheless, the basal cortisol levels were consistently higher for NA/NR pigs than for A/R ones, and this was eventually accompanied by adrenal hypertrophy in the former. The mean heart rate (HR) in beats/min (bpm) was higher of the A/R pigs compared to the NA/NR ones in two backtests. Moreover, in reaction to the novel object (a falling bucket) in the (second) OF HR of the A/R pigs substantially increased (23.9 bpm = 15.5%), while HR of the NA/NR pigs only slightly increased (4.5 bpm = 2.9%). Surprisingly, one-third of the NA/NR individuals even showed a HR decrease (bradycardia) in response to the falling bucket. This implies that the active pigs (A/R) reacted predominantly with a sympathetic response, and the passive pigs (NA/NR) with a parasympathetic one; these findings strongly parallel data found in other animals and humans. The sympathetic response of the active pigs resulted in heart deviations. Thus, active and passive pigs displayed consistent individual differences in behavioural, physiological, and endocrine responses to stress situations leading to different stress pathologies.

Individual immunological characteristics

Chapter 4 reports about individual differences in cell-mediated and humoral immunity as related to different coping styles in pigs. The immune reactivity of 32 A/R and 32 NA/NR pigs was tested in relation to stress using several cell-mediated (CMI) and humoral immunological tests. Results indicated that the active pigs had a higher in vivo and in vitro CMI to non-specific and specific antigens than the passive pigs. Furthermore, in reaction to stressors applied in the present study (i.e., weaning, new environment, transportation, mixing) active pigs had a reduced but temporary CMI response in the first phase of stress, while passive pigs showed a more chronic impairment. In contrast, the passive pigs displayed higher levels of specific antibodies than the active ones. This suggests a converse relationship in the individual pig between CMI and humoral immunity, in that active pigs had a high CMI but a low humoral immunity, whereas passive pigs had a low CMI but a high humoral immunity. This converse relationship may be associated with different levels of glucocorticoids as described in chapter 3. In conclusion, active and passive pigs clearly differed in their immune reactivity to stressful situations.

Practical implications

How far group composition based on the individual coping characteristics may influence the growing up of fattening pigs was tested at a commercial closed farm (cf. chapter 5). During the suckling period, piglets of this farm were individually tested in two successive backtests, and classified as R, NR, or as D. At nine weeks of age, the pigs were grouped into six pens with only R pigs (R pens), six pens with only NR pigs (NR pens), and six pens with both R and NR ones (R/NR pens). The average daily weight gain (ADWG; grams/day) was highest of the pigs in the R/NR pens compared to the pigs in the R pens and in the NR pens. Moreover, the coefficient of variation of ADWG was lower among R/NR pens than among R pens or NR pens. The carcass weight and meat% was somewhat higher and carcass classification was better of the pigs in the R/NR pens than the pigs in the R pens and in the NR pens. Additionally, pigs in the R/NR pens had less pleurisy than the pigs in the other pens, whereas the number of pigs with stomach wall damage was highest for pigs in the NR pens. Groups consisting of both active (R pigs) and passive (NR pigs) individuals seem to better fit each other than groups with only active or with only passive ones and, thus it is worthwhile to compose groups of pigs based on their individual behavioural characteristics. In practice, good management implies besides perfect climatic and feeding conditions also attention for and understanding of the social environment of the farm animals.

General Discussion

In the general discussion three major topics are discussed: 1) do the behavioural differences in pigs represent idiosyncratic response patterns; 2) do the individual behavioural characteristics in pigs relate to different autonomic nervous and immune reactivity under stress conditions and 3) the relevance of applying behavioural studies in pigs in practice. Especially the intriguing finding that under stressful conditions active and passive pigs need each other to develop a stable social organization needs further research.

SAMENVATTING

SAMENVATTING

Inleiding

Het algemene thema van dit proefschrift is individuele gedragsvariatie. Gedragsvariatie tussen individuen binnen een populatie kan informatie geven over alternatieve strategieën, over sociaal gedrag, een persoonlijkheids kenmerk of kan dienen voor individuele herkenning. Deze gedragsvariatie uit zich in het algemeen alleen in stressvolle situaties. Recente gegevens hebben aangetoond dat eigenlijke twee verschillende 'coping' (coping=individuele respons op een stressor waardoor schadelijke fysiologische effecten van deze stressor worden gereduceerd) strategieën bestaan, een actieve dan wel een passieve. Deze verschillende 'coping' stijlen komen overeen met de twee (klassieke) gedragsresponsen tijdens stress; vechten of vluchten versus afwachten of terugtrekken, ieder met een eigen karakteristiek biologisch patroon. Het succes van een individuele 'coping' respons hangt af van de omgevingscondities en daarom is het uitermate verrassend dat ieder individu voorbestemd lijkt te zijn voor de één of de andere copingsstrategie. Dit suggereert een genetische of ontogenetische basis, echter recente ervaringen zijn ook van belang. Een idiosyncratische (=individuele consistentie in een respons op iedere situatie) respons patroon is aangetoond in onder andere mensen, apen, honden en tupajas, en daarom mag worden aangenomen dat dit ook voor varkens geldt. Als dit zo is dan kunnen deze individuele gedragskarakteristieken belangrijke praktische toepassingen hebben zoals het begrijpen van de sociale relaties van groepsgehuisveste varkens in de intensieve omstandigheden van de varkenshouderij. Een stabiele sociale structuur in de groep, en dus een juiste groepssamenstelling, kan een functie zijn van de individuele gedragseigenschappen van ieder afzonderlijk groepslid. Tot op dit moment is echter nog maar weinig onderzoek uitgevoerd om te achterhalen wat de mogelijke patronen zijn voor een juiste groepssamenstelling bij varkens, en verder hoe deze kennis kan worden toegepast in de intensieve varkenshouderij. De studie beschreven in dit proefschrift richt zich op deze aspecten.

Sociale status

In hoofdstuk 1 is de individuele variatie in ziektegevoeligheid en immuunreaktiviteit bij varkens beschreven in relatie met de individuele sociale status in een stabiele sociale groep. Deze sociale status was bepaald middels de uitkomst van sociale rangorde gevechten en voerconcurrentie proeven. De sociale status bepaald middels de sociale rangorde gevechten en voerconcurrentieproeven kwamen goed overeen en omdat deze testen op verschillende leeftijden werden gedaan (respectievelijk; gedurende de zoogperiode en op dag 50, dag 65 en op dag 100) betekent dat er sprake was een relatieve stabiele sociale structuur in de groep. Op 10 weken leeftijd werden alle

varkens kunstmatig besmet met een Aujeszky virus. De sterfte en ziekte was hoger onder de ranglage varkens dan onder de subdominante en de dominante dieren. Een lymfocyten stimulatie test met het Aujeszky virus als een antigene stimulus liet verder zien dat de celgebonden afweer tegen het Aujeszky virus hoger was voor de dominante varkens dan voor de subdominante en ondergeschikte dieren. De resultaten toonden aan dat er grote individuele verschillen bestaan in immuunreaktiviteit en ziektegevoeligheid wat gedeeltelijk gerelateerd was met hun individuele sociale status in de groep. Waarschijnlijk wordt het sociaal gedrag van een dier wat leeft in een sociale organisatie ook bepaald door de individuele manier van omgaan met stressvolle situaties, d.w.z. de copingsstrategie. Daarom kan de individuele copingsrespons wel eens de basis zijn voor verschillende interne biologische programma's die uiteindelijk kunnen leiden tot individuele verschillen in ziektegevoeligheid. In hoofdstuk 2 is onderzocht of er consistente individuele gedragseigenschappen bij varkens bestaan.

Individuele gedragseigenschappen

In de zoogperiode werden de biggen geklassificeerd door twee waarnemers als agressieve (A) of als niet-agressieve (NA) dieren in twee sociale confrontatie (SC) testen. Er bestond grote overeenkomst in deze classificatie niet alleen tussen de twee waarnemers maar ook tussen de twee SC testen. Bovendien waren de A varkens opnieuw de meest agressieve dieren na het mengen op 10 en 15 weken leeftijd. Dit betekent dat het individuele gedragspatroon van varkens consistent was over een lange tijdsperiode. In de niet-sociale rugtest werden de biggen individueel op hun rug gelegd en voor 60 seconden zo vastgehouden. De biggen konden worden geklassificeerd in verzetters (R; > twee ontsnappingspogingen), in intermediair (I; twee ontsnappingspogingen) en in niet-verzetters (NR; < twee ontsnappingspogingen). Op basis van vijf opeenvolgende rugtesten in week 1, 2 en 3 werden de biggen geklassificeerd als R (n=95), NR (n=77) of als Twijfel (D; n=46). De resultaten gaven aan dat twee rugtesten voldoende is voor een praktische toepassing. Een verrassende bevinding was dat er een sterke associatie bestond tussen de uitkomst van de rugtest en die van de SC testen. Namelijk de verzetters in de rugtest waren ook de agressieve individuen in een sociale situatie, terwijl de niet-verzetters de niet-agressieve dieren waren. Een dergelijke associatie en ook de sterke consistentie over de tijd suggereert een individuele gedragsstrategie om om te gaan met conflict situaties. Deze idiosyncratische eigenschappen impliceert een bimodale verdeling in copingsgedrag bij varkens; er zijn actieve (agressief/verzetters; A/R) of passieve (niet-agressief/niet-verzetters; NA/NR) varkens.

Individuele fysiologische eigenschappen

Hoe deze individuele gedragsstrategieën in varkens zijn gerelateerd aan verschillende gedrags-, fysiologische- en endocrinologische responsen in stressomstandigheden is beschreven in hoofdstuk 3. Hiervoor zijn 32 A/R (actieve) en 32 NA/NR (passieve) varkens geselecteerd en individueel getest in een "open field" (OF) test op 3 en op 8 weken leeftijd. Meer A/R dan NA/NR varkens probeerde te ontsnappen uit de OF, en de A/R individuen vocaliseerden gedurende de OF-procedure ook meer dan de NA/NR dieren. Verder exploreerden de A/R varkens een nieuw object in de OF (kartonnen doos (OF1) en een emmer (OF2)) snel maar kort, terwijl de NA/NR dieren dit langzaam maar intensief deden. De cortisol respons op de OF (t=0/t=90) was verschillend tussen de beide typen varkens. De cortisol respons op een farmacologische dosis $ACTH_{1-39}$ (2,5 IE/kg lichaamsgewicht per varken) op 3 en 8 weken leeftijd was niet significant verschillend tussen de beide typen varkens. Desalniettemin waren de basale cortisol gehaltes consistent hoger in de NA/NR varkens dan in de A/R dieren, en dit ging gepaard met een bijnier hypertrofie in de NA/NR varkens. De gemiddelde hartslag gemeten in twee rugtesten was hoger in de A/R varkens dan in de NA/NR dieren. Bovendien steeg de hartslag van de A/R varkens sterk (23.9 hartslagen = 15,5%) in reaktie op het nieuwe object (een vallende emmer) in OF2, terwijl de hartslag van de NA/NR dieren nauwelijks omhoog ging (4,5 hartslagen = 2,9%). Verrassend was dat bij 1/3 van de NA/NR varkens de hartslag zelfs significant omlaag ging (bradycardia). Dit suggereert dat de actieve varkens voornamelijk reageerden met het sympathische gedeelte van het autonome zenuwstelsel terwijl de passieve dieren meer reageerden met het parasympatische gedeelte. De sympathische reaktie van de actieve (A/R) varkens resulteerden in hartafwijkingen. Dus actieve en passieve varkens vertoonden consistente individuele verschillen in gedrag, fysiologie en endocriene responsen op stress situaties wat kan leiden tot verschillende stresspathologiën.

Individuele immunologische karakteristieken

Hoofdstuk 4 beschrijft de individuele verschillen in celgebonden en humorale immuniteit gerelateerd aan verschillende copingstijlen bij varkens. De immuunreaktiviteit van de 32 A/R en de 32 NA/NR varkens in relatie met stress was getest met behulp van verscheidene celgebonden en humorale immunologische testen. De resultaten gaven aan de actieve varkens een hogere in vivo en in vitro celgebonden immuniteit op niet-specifieke en specifieke antigenen hadden dan de passieve dieren. In reaktie op de stressoren in dit experiment (spenen, nieuwe omgeving, verplaatsen en mengen) toonden de actieve varkens een gereduceerde maar kort durende

celgebonden immuunrespons in de eerste fase van de stress, terwijl de passieve varkens meer een chronische verlaging lieten zien. In tegenstelling vertoonden de passieve varkens hogere gehaltes van specifieke antilichamen dan de actieve dieren. Dit suggereert een tegengesteld verband in het individu tussen de celgebonden en de humorale immuniteit; actieve varkens hadden een hoge celgebonden maar een lage humorale immuniteit terwijl de passieve varkens een lage celgebonden maar een hoge humorale immuniteit hadden. Concluderend, actieve en passieve varkens verschilden duidelijk in hun immuunreaktiviteit in stressvolle situaties.

Praktische toepassing

Hoe een groepssamenstelling, gebaseerd op de individuele copingeigenschappen, het opgroeien van de vleesvarkens kan beïnvloeden is getest op een gesloten varkensbedrijf. Gedurende de zoogperiode werden twee rugtesten uitgevoerd, en de biggen werden geklassificeerd als R, NR of als D. Op 9 weken leeftijd werden zes hokken met alleen R, zes hokken met alleen NR en zes hokken met zowel R als NR varkens geformeerd. De gemiddelde groei (gram/dag) was hoger van de varkens in de R/NR hokken dan in de R hokken en NR hokken. De variatiecoefficient van de gemiddelde groei was lager in de R/NR hokken dan in de andere hokken. Het karkasgewicht en het vlees% was enigszins hoger en ook de karkasclassificatie was beter van de varkens in de R/NR hokken dan van de varkens in de R en in de NR hokken. Het aantal varkens met borstvliesontsteking was in de R/NR hokken ook minder dan in de andere hokken, terwijl het aantal varkens met maagwandbeschadigingen het hoogste was in de NR hokken. Dus is het voordelig om groepen varkens samen te stellen gebaseerd op de individuele gedragseigenschappen. Verder passen schijnbaar groepen varkens bestaande uit zowel actieve (R) als passieve (NR) dieren beter bij elkaar dan groepen met alleen actieve of alleen passieve dieren. In de praktijk is het belangrijk om naast perfecte klimatologische- en voedingsomstandigheden en een goed management ook oog te hebben voor en het begrijpen van de sociale omgeving van de landbouwhuisdieren.

Algemene discussie

In de algemene discussie worden drie items besproken; 1) zijn de individuele gedragseigenschappen in varkens idiosyncratische responsen; 2) zijn de individuele gedragseigenschappen gerelateerd aan verschillende autonomische zenuw- en immuunreaktiviteit onder stressomstandigheden, en 3) het belang van de toepassing van gedragsstudies bij varkens in de praktijk. Vooral het intrigerende feit dat onder stressomstandigheden de actieve en passieve varkens elkaar nodig hebben om tot een stabiele sociale organisatie te komen verdient verder onderzoek.

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DANKWOORD

Het algemene thema van dit proefschrift is individuele gedragsvariatie bij varkens. Een dergelijke variatie geldt zeker ook voor al die individuen die een belangrijke bijdrage hebben geleverd bij het tot stand komen van dit proefschrift, natuurlijk niet suggererende dat al deze individuen varkens zijn. Toch wil ik een paar van die ~~varkens~~, ehh individuen met name noemen:

Teneerste mijn promotor, Piet Wiepkema. Beste Piet, jouw heldere kijk op onderzoek en een kritische noot op de momenten waar nodig, zijn van onmisbare waarde gebleken. Je teleac cursus "hoe schrijf ik een wetenschappelijk artikel" heb ik altijd met interesse gevolgd; ik heb veel van je geleerd.

Mijn tweede promotor, Martin Tielen. Beste Martin, ik wil je bedanken voor de mogelijkheid die je me hebt gegeven om in de Klimaatstal 't vak **varkensverzorger** te leren. Het feit dat toegepaste ethologie toch zijn weg vindt naar de praktijk is mede jou verdienste en namens de landbouwhuisdieren dank daarvoor.

Een gedragsfysioloog met kennis van de praktijk, mijn co-promotor Willem Schouten. Beste Willem, jouw interesse en enthousiasme voor het individuele varken heeft van dit proefschrift iets extra gemaakt. Zonder jouw inzet zouden een aantal fysiologische parameters onderbelicht zijn gebleven.

Naast dit illustere trio zijn vele instanties op velerlei wijze betrokken geweest bij de uitvoering van het onderzoek zoals de Gezondheidsdienst voor Dieren in Zuid-Nederland. Alle individuen van het laboratorium, buitendienst, sektiezaal, technische dienst, huishoudelijke dienst en secretariaat wil ik hartelijk bedanken voor al het werk wat is verzet.

Zonder hardwerkende individuen die vaak aanwezig moesten zijn op de meest onmogelijke tijden (ook wel stagiaires genoemd) zou 't onderzoek niet mogelijk zijn geweest. Els Daniëls, Wim Drossaert, Anita Donkers, Janet Beekman, Paula Hamm en Carl van Doorn bedankt voor jullie inzet.

Mijn onderzoekstijd op het "National institute of Animal Science, research centre Foulum" in Denemarken zal ik nooit vergeten. De gastvrijheid van de Deense individuen en de onderzoeksmogelijkheden daar was ongekend. Mette Hagelsø, Gerard Coenen, Anna Halborg Madsen, Poul Henkel, Rie Krukow, the personnel of the piggery and all individuals housed in the apartments nearby the institute, thanks for your extensive help in the experiments, but especially for making my stay in Denmark a very pleasant one. In dit rijtje hoort met name ook Jan van Beek, een Nederlands individu in Denemarken, thuis. Jan bedankt voor al je hulp, zelfs het bloedtappen had je al snel in de vingers.

Mijn dank gaat ook uit naar twee individuen van de sectie Immunologie in Wageningen; Mike Nieuwland en Henk Parmentier bedankt voor jullie immunologisch gebrabbel.

Behalve in Brabant is het leven in het Limburgse ook uitstekend vooral bij de familie Jenniskens, bedankt voor de gastvrijheid!

Naaste familie, vrienden en andere bekende individuen waren altijd geïnteresseerd in het wel en wee van de varkens. Alleen als ik het had over het castreren van biggen zat menig mannelijk individu wat bedenkelijk te kijken.

Op 22 februari is 't dan zover, de verdediging van mijn proefschrift maar gelukkig sta ik er niet alleen voor. Mijn paranimf, Kees Scheepens. Beste Kees, onze discussies omtrent het gedrag, welzijn en gezondheid van het varken zijn belangrijk voor het onderzoek geweest en als een ieder stelling 5 ter harte neemt moet "Outdoor Pig Production" ook in Nederland een kans maken. Mijn paranimf en belangrijkste schakel van het onderzoek, Mariëtte Prince. Lieve Mariëtte, jouw liefde en steun, maar vooral je enthousiasme voor het varken is **de stimulans** voor mij geweest. Hoewel je niet zo van slachthuizen en sektiezalen houdt kun je als de beste bijnieren vetvrij maken. Mocht ik gedurende de verdediging onverhoopt flauwvallen kun jij het zonder problemen overnemen. Bedankt Snoepie!

CURRICULUM VITAE

Manfred Jacobus Cornelis Hessing werd geboren op 20 november 1963 in Son en Breugel (NBr). In 1982 behaalde hij het VWO diploma aan de Gemeentelijke Scholengemeenschap Woensel in Eindhoven. In september van datzelfde jaar begon hij met de studie Zoötechniek aan de toenmalige Landbouwhogeschool in Wageningen. In juni 1988 studeerde hij af aan de Landbouwuniversiteit Wageningen met als afstudeervakken Gezondsheids- en Ziekteleer en Ethologie.

Gedurende een jaar werkte hij mee aan een onderzoeksproject "Effect van tocht op de gezondheidstoestand van gespeende biggen" een samenwerkingsverband van de Gezondheidsdienst voor Dieren in Zuid-Nederland, de vakgroep Bedrijfsdiergeneeskunde & Voortplanting van de Rijksuniversiteit Utrecht en de sectie Ethologie van de vakgroep Veehouderij van de Landbouwuniversiteit Wageningen.

Op 15 augustus 1989 volgde de aanstelling als Assistent in Opleiding bij de sectie Ethologie van de vakgroep Veehouderij van de Landbouwuniversiteit Wageningen dat resulteerde in dit proefschrift. Vanaf 1 oktober 1993 is hij werkzaam als nutritionist pluimvee bij UTD.