PRODUCTION STRATEGY OF FUNCTIONAL FOODS OF ANIMAL ORIGIN

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SUMMARY

The procedures implemented in the production of functional foods of animal origin should be based on the scientifically verified new knowledge comprising the entire food chain. Possible physiological and psychological effects of such foods on humans should not be neglected. Although the human genome did not change since the Palaeolithic, however the nutritional habits and way of life of modern people have changed significantly and subsequently led to the development of various chronic degenerative diseases. Fatty acids, in particular n-3 unsaturated acids, are important, biologically active molecules characterised by a series of positive effects in the prevention and improvement of both human and animal health. Some of the modes of increasing the intake of n-3 unsaturated fatty acids include the increase of content of these fatty acids in feed fed to farm animals or in rearing of animals of more advantageous genotype. Irrespective of the method used for increasing the intake of advantageous fatty acids, care should always be taken about the animal welfare and health, because of different basic needs of animals under stress for individual nutritive components, among which n-3 unsaturated fatty acids are especially important.

This paper presents some of possible methods for increasing the content of n-3 unsaturated fatty acids in meat and other products of animal origin, which could meet the criteria set for the functional foods.

***Key words**: Animal rearing, functional food, health, n-3 unsaturated fatty acids*

INTRODUCTION

In recent years, in the field of both food and feed science a special attention has been paid to the understanding of the role of individual food ingredients as modulators of the body functions, which are either responsible for maintaining and promoting health and welfare, or participate in the prevention and reduction of risks of the increasing occurrence of chronic diseases in the population. Foods not only help to support different life important processes in the human body, but also create the feeling of contentment. This particularly refers to the increasing belief among the consumers about the important role of functional foods in the protection against certain diseases and their health beneficial properties. In the Western civilisation, food is no more considered solely as means for mere survival and appeasement of hunger. Individual food ingredients are declared as «functional» according to the criteria set by the health claims legislation. Accordingly «any food can be regarded as functional if it can be demonstrated to have beneficial effect on one or more target functions in the body or reduce disease risk besides basic nutrition» (Bellisle et al., 1998). According to Clydesdale (1997), functional food has a physiological or psychological connotation beyond its traditional effect. Food related regulations established by the European Union state as follows: «health claim means any claim that states, suggests or implies that a relationship exists between food category, a food or one of its constituents and health» (Regulation EC, No 1924/2006), while «structural or functional claim refers to the benefit of any food ingredient in relation to disease» (Balenović and Pollak, 2007).

Development and manufacture of functional foods presents a scientific challenge that must precede the economical one, and should be based on scientifically verified steps as presented in Figure 1. There are several directions for reaching the target areas of the functional food development, such as: gastrointestinal tract, redox and anti-oxidative system, metabolism of macronutrients, early foetal development and life of newborns, metabolism of xenobiotics, physical activity, mood and mental abilities. Animal production is also an important field in the development of functional foods, especially the production of meat, eggs, milk and dairy products. When farm

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animals are concerned, the production of functional foods is primarily associated with the nutrition management, which makes possible to either increase or decrease the ratio of biologically active components in their products and excretion. It is important that each animal category is given adequate feed, which will produce beneficial effects on the animal welfare and health, and indirectly also, on human health. This article deals with only a part of research in the field of functional foods, which refers to polyunsaturated fatty acids, the source of which could be different products of animals specifically fed or selected. The purpose is to increase the content of n-3 unsaturated fatty acids in food products by using biologically active macromolecules for the reduction of risks of cardiovascular and metabolic disease in humans.

FATTY ACIDS, NUTRITION AND SELECTION

Based on our own novel knowledge and literature data, it may be concluded that the introduction of a definite practice in the nutrition and breeding of production animals could increase the content n-3 unsaturated fatty acids and other suitable fatty acids in meat, milk and eggs. Consequently, positive results could be attained within a short period, provided the adoption of a defined strategy of animal nutrition is applied. In recent years, a large number of researchers have shown great interest in the chemical composition of animal food products, especially of certain components such as fatty acids because of their impact on human health. A crucial fact concerning the altered ratio of n-6/n-3 unsaturated fatty acids in human nutrition has become a matter of great concern. Significant increase of the ratio in favour of n-6 unsaturated fatty acids was the result of the Western mode of life and nutrition in which fish and vegetables were less and less consumed. Identical sequence occurred in case of fat content and composition of fatty acids. Intensive animal breeding and mode of feeding under modern technological conditions have often replaced free-range rearing of animals with access to pasture grazing. Consequently, the experts in animal management and nutrition were faced with new challenges, which indirectly concerned also the human wellbeing and health.

The aim of a large number of conducted studies was to develop an adequate method of the production animal nutrition in order to obtain meat with a desired ratio of unsaturated and saturated fatty acids (U/S) at the recommended levels (not more than 0.7), and the n-6/n-3 ratio less than 5. Considering the known efficacy of some n-3 unsaturated fatty acids, eicosapentaenoic and docosahexaenoic acid (EPA; C20: 5n-3 and DHA; C22: 6n-3) their ideal ratio in diet is currently under investigation; approx. 1.6 is presumed to be the optimum ratio. This ratio is considered to be most efficacious in the prevention of disorders induced by inflammatory conditions, e.g. arthritis and systemic lupus erythematosus, as well as of the development of atherosclerosis and some malignant processes (Bhattacharya et al., 2007). The recommended daily dose of EPA+DHA is 0.45 g, but the needs increase in the last third of pregnancy and during lactation period. Recent discoveries suggest that individual metabolic disorders, like osteoporosis, are also associated with the increased daily intake of n-6 in comparison with n-3 unsaturated fatty acids (Weiss et al., 2005). Alteration of their unfavourable ratio in human diet and animal nutrition becomes an imperative. The ultimate objective is to reduce the content of linolic acid (LA), and increase both the content of linolenic acid (LNA) and direct attainment of long chain polyunsaturated fatty acids (LCPUFA).

Daily intake of n-3 unsaturated fatty acids can be directly increased, by enrichment of foods of animal origin with unsaturated fatty acids, or indirectly by implementation of a defined strategy of animal nutrition. Possibilities of selection and breeding of animals with improved meat fat ratio and fatty acid composition have been widely discussed (Fernández et al., 2002, Knight et al., 2004; Karamichou et al., 2006). According to the finding of De Smet et al. (2004), the ratio of unsaturated to saturated fatty acids is mostly affected by genetic factors. On the other hand, much more important for human health is the ratio of n-6 to n-3 unsaturated fatty acids, which depends on dietary composition of fatty acids. By increasing the content of n-3 unsaturated fatty acids in feed fed to breeding animals resulted in increased intramuscular fatty acid composition and simultaneous reduction of n-6 unsaturated fatty acids. It should be pointed out that muscular fat and fatty acids are located within and between the muscle fibres. Also, fat in the intramuscular fatty tissue is stored in fat cells that are either isolated or accumulated in groups along the muscle fibres, but also in the inter-fascicular sites. Fat is mostly composed of triacylglycerol (TAG), phospholipids and cholesterol. Knight et al. (2004) claim, based on their studies and contrary to expectations, that the TAG is prevailingly present in loci between rather than within the muscle fibres and that the TAG composition shows more expressed heredity compared to the composition of phospholipids, in spite of their life-important significance for both the cell and entire body. According to their estimation, the genetic index in beef cattle for myristic and palmitic acid is 0.49, 0.40 respectively, and it exceeds 0.5 in case of palmitoleic and oleic acids. Efforts have been made to develop DNA markers for application in animal selection in order to improve the ratio of fatty acids in beef (Zhang et al., 2008). In case of cattle, there are enough possibilities to meet the consumers' requirements by selection, because of a large variability for most traits, both within and between breeds. For example, Štoković et al. (2007) found 3.12% of fat with a markedly high variability (CV%=51.16%) in *m. longissimus dorsi* in the Croatian Simmental bulls (KV%=51,16%). Results of scientific researches show that the deposits of unsaturated fatty acids increase with the animal age (Fries and Ruvinsky, 1999).

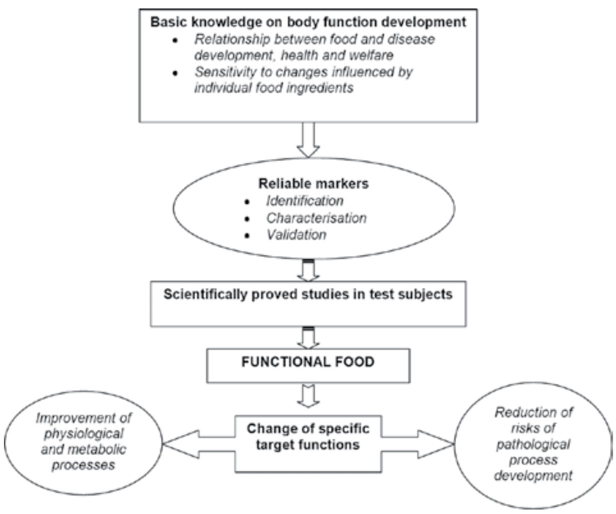
Comparison of the effect of feeding and genetic selection on fat content and fatty acid composition of triacylglycerols and phospholipids in pork has shown a significantly higher effect of diet. This effect is primarily evident with respect to n-6 to n-3 ratio of unsaturated fatty acids, but the genotype with nutrition interactions were not detected (Cameron et al., 2000). Nutrition-induced genotype changes would necessitate a longer period of time, as observed in similar studies carried out in salmons (*Salmo saler*) (Thomassen, 2007). As the composition of fatty acids in triacylglycerols changes primarily under the influence of nutrition, the same occurs with the composition of fatty acids in the intramuscular fatty tissue. Furthermore, as regards the fatty acid composition, a difference was also found in pigs with respect to sex in which the content of PUFA in total lipids was higher in female in comparison with male animals (Cameron et al., 2000). It is also important to point out that PUFA are mostly found in phospholipids in the amount of 20-50% in relation to total content of fatty acids, and that oxidative muscles contain more mitochondria, and consequently more PUFA. The content of PUFA in the composition of phospholipids is influenced by the complex elongase and desaturase enzymatic system that is responsible for the conversion of LA and LNA into long chain polyunsaturated metabolites. Animals susceptible to stress have a significantly higher U/S ratio in the muscle and fat tissue. Stress from overcrowding, especially in case of fattening animals, is considered by some researchers to be a possible cause, besides the nutritive factors, of increased occurrence of fatty liver degeneration and liver infiltration (Gazdzinski et al., 1994). Effects of high n-3 unsaturated fatty acid level in feed given to those animals could result in positive ability of regeneration and normal development of cell functions (Otten et al., 1997). Consequently, the exposure to stress significantly increases the animal needs for n-3 unsaturated fatty acids.

CONTENT OF FATTY ACIDS IN INTRAMUSCULAR FAT AND EFFECT OF ADDITION OF N-3 UNSATURATED ACIDS ON THE PRODUCT'S RATIO OF FATTY ACIDS

Nutrition of animals with fodder or mixes enriched with n-3 unsaturated fatty acids will change the composition and ratio of fatty acids depending on the animal species and type of fat deposits. It is not all the same whether it concerns subcutaneous, intermuscular or intramuscular fatty tissue. Table 1 presents the content of fatty acids in meat of animals given the standard feed and those fed feed rich in n-3 unsaturated fatty acids. Beef is characteristic for its lower content of intramuscular fat with a higher level of polysaturated fatty acids, while subcutaneous tissue has a higher content of saturated fatty acids (SFA). Considering the abovementioned, and also the mode of the slaughtering procedure of removal of beef subcutaneous adipose tissue, the intake of SFA is low. On the other hand, the intake of pork fat refers to the inter- and intramuscular and subcutaneous fat. The basic difference between the composition of subcutaneous and intramuscular fat is in the content of PUFA that is higher in the intramuscular fat. Karolyi (2007) has reported that fatty breeds of pigs, e.g. Black Slavonian breed and Iberian pig, used in the production of smoked ham, have a higher content of intramuscular fat in comparison with modern breeds and hybrids. In addition, the LA/LNA ratio does not differ significantly with regard to the type of fat deposit, but it differs with regard to the animal species. Beef has a higher content of LA and LNA in intramuscular fat, and pork in subcutaneous fatty tissue.

It has been established so far that by the type of nutrition in monogastric animals it is much easier to attain targeted changes in the composition of fatty acids in fat deposits and intramuscular adipose tissue in comparison with ruminants. However, regardless of specific processes in the complex alimentary system of ruminants, their meat, and in particular milk, are inclined to significant changes in the fatty acid composition (Marenjak et al., 2008). These changes depend on the nutritional method applied (Chilliard et al., 2001; Bauman and Griinari, 2003) and use of specific feed additives (Palmquist and Griinari, 2001; Marenjak, 2006), and are accompanied by changes in the blood lipid fractions (Marenjak et al., 2008). Poultry are also very suitable for nutritional studies in which the planned effect is rapidly attained, and it is relatively easy to

▼ **Figure 1.** Scientifically confirmed criteria in the functional food development



obtain increased content of PUFA in meat and egg yolk.

There are different sources of n-3 unsaturated fatty acids suitable for animal nutrition. Increased content of linolenic acid (LNA) will be attained just with the use of additives of vegetable origin, linseed or linseed oil and oil rapeseed. Similar effect is attained when feeding ruminants with a specific grass mixture or hay (Marenjak and Poljičak – Milas, 2005). With the addition of vegetable oil with a higher level of LNA in feed, especially in feed fed to monogastric animals and poultry, it is possible to expect the increased content of n-3 unsaturated fatty acids in muscular tissue and eggs. The additive containing linseed oil with almost 53% of LNA was mostly used in different studies. In studies performed in pigs the addition of linseed oil in feed resulted in an increased LNA level and deposition of EPA and docosapentaenoic acid (DPA; C22: 5n-3) in intramuscular adipose tissue, but with no changes in the DHA concentration (Fontanillas et al., 1997). Conversely, Rey et al.(2001) has reported the increased DHA level after the addition of a smaller quantity of linseed oil, although the basic ration contained also olive oil. The finding confirmed that the addition of only 0.5% of linseed oil suffices for altering the fatty acid composition of intramuscular adipose tissue. Linseed oil treated with formaldehyde was used in studies in beef cattle for the reduction of protein degradation and bio-hydrogenation of fatty acids in the rumen (Choi et al., 2000). Increase of n-3 unsaturated fatty acids was confirmed, particularly as regards LNA, EPA, DPA, but not DHA. According to Raes et al. (2004), the animal nutrition in the early period of life is of exceptional importance for the formation and incorporation of long chain PUFA in intramuscular adipose tissue, especially in phospholipids. Continuous, long-term feeding with specially adapted diet is of utmost significance. Increased level of n-3 unsaturated fatty acids in intramuscular adipose tissue was found in beef cattle kept on pasture in comparison with animals given concentrated feed. Six-fold increases of EPA and LNA levels and a three-fold increase of DHA were recorded in meat (Lorenz et al., 2002). In free-range rearing of pigs with access to

▼ **Table 1.** Standard (S) and experimental (n-3) values of fatty acids content in foods of animal origin (data partially obtained from the work of De Henauw et al., 2007)

	Fatty acids, %											
	SFA[a]		MUFA[b]		C18:2n6 (LA[c])		C20:4n6 (AA[d])		C18:3n3 (LNA[e])		LCn-3 (EPA[f] + DPA[g] + DHA[h])	
Fat origin	S	n-3	S	n-3	S	n-3	S	n-3	S	n-3	S	n-3
Beef	39	38	39	38	8,5	8,5	1,5	1,5	1	2	0,5	1
Pork	39	37	39	37	14	14	0,4	0,4	1,5	5	0,3	0,5
Poultry meat	30	28	38	36	17	14	3	3	2,5	7	2	4,5
Milk	68	58	25	32	1,5	3	0	0	0,5	1,3	0	0
Eggs	31	30	42	39	20	18	2	1	1	7	1,5	2,5
Lamb	36	-	35	-	6	-	2,2	-	1,5	-	2,8	-
Horse meat	36	-	34	-	13	-	1,7	-	7,5	-	1,6	-
Rabbit meat	37	28	30	31	20,4	18,5	0,02	0,60	5,51	17,5	1,32	1,32
Game												
Dear	36	-	13	-	22,3	-	10,7	-	2,3	-	6,7	-
Wild Boar	35	-	46	-	12,6	-	1,94	-	1,22	-	0,48	-
Wild duck	35	-	23	-	22	-	15	-	1,0	-	3,13	-
Hare	34	-	17	-	34	-	8,1	-	1,36	-	3,25	-
Hare*	49	-	28	-	15,3	-	ND	-	8,3	-	ND	-

**m. psoas major*- results form our own study (unpublished data); [a]SFA - saturated fatty acids; [b]MUFA - monounsaturated fatty acids; [c]LA – linoleic acid; [d]AA – arachidonic acid; [e]LNA- linolenic acid; [f]EPA- eicosapentaenoic acid; [g]DPA- docosapentaenoic acid; [h]DHA- docosahexaenoic acid; [i]LC – long chain fatty acids; ND- not detected pasture grazing and fed a combination of silage and concentrates also had a higher content of n-3 unsaturated fatty acids in intramuscular adipose tissue in comparison with conventionally grown pigs given only the concentrate feed (Nilzen et al., 2001). Similar results were recorded in meat of rabbits grown under ecological conditions (Pla et al., 2005). For the purpose of increasing the content of n-3 unsaturated fatty acids, different fish and other sea products, fish oil or fish meal and marine algae oil are often added to feed. Possibility of production of genetically modified oleiferous plants and cereals by using the genes of marine algae is widely discussed. Presumably, it could increase the content of DHA in their fatty acid composition, and thus also in the daily diet of consumers. This is one of the research priorities of an extensive and highly valuable project of the European Union, which is based on the studies of Tonon et al. (2003, 2005).

Investigation of a single segment, such as animal nutrition, is just a part of the extensive research activities undertaken for the purpose of increasing the content of n-3 unsaturated fatty acids in the food chain. There is a significant potential in specially adapted nutrition of dairy cows leading to changes in the content of individual fatty acids in milk fat and meat of calves in the cow-calf production system. Changes occurring due to the microflora activity in the rumen are a key factor of the altered fatty acid composition of meat and milk fat. Biohydrogenation of n-3 unsaturated fatty acids in the rumen is limited. Increased contents of PUFA and conjugated linoleic acid (CLA; *cis*-9, *trans*-11 C18:2n-7) have been recorded in cow's milk produced after the addition of fish oil in feed (Palmquist and Griinari, 2001), or when kept on mountain pastures (Collomb et al., 2002). In addition, the content of fatty acids in milk can be influenced by quantity, mode of intake and access to feed, limited administration of certain feed respectively (Marenjak and Poljičak-Milas, 2005). According to the study results, (Jiang et al., 1996), changes in dietary regimen, i.e. reduced quantity of green forage in relation to concentrate feed, resulted in by 30% higher CLA milk concentration. The respective changes are evidently associated with the supply of substrate and microbiological alteration in the ruminal content. The interaction of basic dietary lipids and fish oil occurs after the addition of fish oil in feed. A marked increase of *trans*-C18:1, primarily of *trans*-vaccenic acid (TVA) in milk in relation to moderate increase of LA, has been explained by inhibitory effect of fish oil in the last phase of biohydrogenation, in the first place on biohydrogenation of LA and LNA from the basic diet (Palmquist and Griinari, 2001). The addition of marine algae oil to feed fed to cows will result in significantly increased levels of DHA and DPA, and also arachidonic acid (AA; C20:4n-6), with the exception of EPA. Results of the studies in pigs given feed with the addition of fish oil or fish meal also showed increased EPA and DHA levels in intramuscular adipose tissue, but with no increase in DPA. Similar results were obtained in studies in lambs and beef cattle in which a two-fold increase of EPA and DHA levels were recorded in *m. longissimusu dorsi* after the addition of fish meal in feed.

Since it is rather difficult to change the human nutritional habits, and there are also disputes regarding the consumption of fish species rich in n-3 unsaturated fatty acids, that are also on the top position of the food chain with a higher accumulation of harmful substances present in sea water, it would be useful to change the strategy of nutrition in the farm animals. That would enable to obtain the nutritional value of fatty acid composition in foods as close to the recommended criteria as possible. It could be concluded that by increasing the content of n-3 unsaturated fatty acids in feed inevitably influences both the accessibility and intake of foods of appropriate fatty acid composition, and thus contributes to the prevention of the increasing occurrence of chronic diseases.

ZUSAMMENFASSUNG

STRATEGIE DER HERSTELLUNG VON FUNKTIONALER NAHRUNG ANIMALER HERKUNFT

Die Herstellung der funktionalen Nahrung animaler Herkunft verlangt Verfahren, die auf wissenschaftlich bestätigten Erkentnissen gründen und die die gesamte Ernährungskette umfassen. Dabei dürfen sowohl die physiologischen als auch die psychologischen Wirkungen, die eine solche Nahrung auf die Menschen haben könnte, nicht vernachlässigt werden. Mit Bezug darauf, dass sich das menschliche Genom seit Paläolithik nicht änderte, aber dass sich die Ernährungs- und die Lebensgewohnheiten der Menschen der modernen Zeit weit von dieser Zeitspanne entfernt haben, entwickelten sich chronische degenerative Krankheiten. Fettsäuren, besonders die n-3 ungesättigten Säuren, sind wichtige biologisch aktive Moleküle, denen eine Reihe von positiven Wirkungen bei Prävention und Verbesserung der Gesundheit bei Menschen und Tieren zugeschrieben wird. Einige der Module, die zur Vergrößerung der Einnahme von n-3 ungesättigten Säuren benutzt werden, sind: Vergrößerung der Anteile der angeführten Fettsäuren im Futter der Tiere auf den Farmen oder die Zucht der Tiere des erwünschten Genotypus. Ohne Rücksich auf die Einnahmeart der erwünschten Fettsäuren, dürfen das Wohlbefinden und die Gesundheit der Tiere nicht vernachlässigt werden, weil die Tiere, die dem Stress ausgesetzt werden, schon grundlegend andere Bedürfnisse mit Bezug auf die Einnahme von einzelnen Nahrungselementen haben werden, von denen die n-3 ungesättigten Fettsäuren außerordentlich wichtig sind.

Schlüsselwörter: *Tierzucht, funktionelle Ernährung, Gesundheit, n-3 ungesättigte Fettsäuren*

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Received: July, 17, 2008
Accepted: August, 2, 2008

HORSEMEAT AND HIPPOPHAGIA

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SUMMARY

Horsemeat represents special and valuable food. An increasing trend in the production of horsemeat in the world is noticeable. Within the EU countries, the average consumption of horsemeat per capita is 0.4 kg per year, but, because of their own insufficient production, they cover 66.7% of the market demand by import.

A higher content of water, proteins and glycogens, and a smaller content of fat in horsemeat make it more suitable for nourishment, particularly of more demanding people, in comparison to pork or beef.

The geographic position and the breed structure of the horse population in Croatia provide good chances for a profitable production of horsemeat with possible export orientation.

INTRODUCTION

The production of horsemeat in the world shows a slight upward trend with insignificant oscillations. In Croatia, total annual production in 1999 amounted to 581,000 t in comparison to 482,000 t in 1989 (FAO Production Yearbook). In 2002, in 14 biggest producers of horsemeat in the world about 700,000 t were produced, most of which in China, Mexico, Kazakhstan, Italy, Argentina and Mongolia. In 2001, the horsemeat production in Europe was over 153,000 t. The biggest producers were Italy, France, Belgium, Netherlands, Spain and Germany. However, the consumption per capita in Europe shows a slight drop, but after the stagnation in the mid ’90-s of the last century, the consumption started to grow slowly. Within the EU countries, the average annual consumption of horsemeat per capita is 0.4 kg, with the exception of Italy, one of the leading countries in the consumption of horsemeat, where it is 1.3 kg (Martuzzi et al., 2001). There is a certain pattern in the seasonal trends of consumption, which coincide with the season of weaning of foals, and consequently, the highest consumption is recorded during winter months. The lowest consumption is during the summer because of a reduced supply of this type of meat (Manfredini and Badiani, 1993). The production of horsemeat in the West European countries is insufficient, so that 66.7% of the market demand is covered by import (Martin-Rosset, 2001). The term *hippophagia* is an obsolete, forgotten name used for horsemeat, consumption of meat of equidae respectively, and it originates from the Greek words: hippos= horse and phagein = to eat.

BREEDING OF HORSES FOR MEAT PRODUCTION

The production of horsemeat is based on an accurate evaluation of the relevant market indicators and ecological conditions under which this production is carried out, and also on the right selection of genotypes as the basis of production. Current production systems are in general of extensive type, in which waste, marginal and less cultivated pasture areas are used for grazing of more primi-

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