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Kaposvári Egyetem, Állattudományi Kar, Kaposvár

University of Kaposvár, Faculty of Animal Science, Kaposvár



## **Acta Agraria Kaposváriensis**

Az Acta Agraria Kaposváriensis évente több alkalommal megjelenő tudományos folyóirat, amely eredeti tudományos közleményeket, kutatási eredményeket, kritikai összefoglalókat, konferenciákról beszámolókat, könyvismertetéseket és szerkesztőhöz küldött leveleket közöl a mezőgazdaság, elsősorban az állattenyésztés és az állati termék előállítás minden területéről.

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**PROCEEDINGS  
OF THE  
18<sup>TH</sup> INTERNATIONAL SYMPOSIUM  
“Animal Science Days”**

**POSSIBILITIES AND DELIMINATIONS OF  
EXTENSIVE ANIMAL HUSBANDRY**

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# **PLENARY SESSION**





## Extensive versus modern animal husbandry – pros and cons

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### **ABSTRACT**

*Food industry has significantly started affecting eco-system and it is more and more clear that we cannot only produce. It has become more important how we produce, as well as how to produce food with economical usage of raw materials and energy with respect to the environment. Nowadays, we face many facts that prove the intensive animal husbandry (IAH) is not so modern anymore and that we have to search back for certain elements from the extensive animal husbandry (EAH) in order to reactivate them in cost-efficient way and with regard to preserving biodiversity, rural development, protection of natural heritage, traditions and animal welfare. It has been identified that upraising of EAH in the future depends much on land support, land-use management, climate, economical development of each country, education and motivation of farmers, as well as on the possibilities to be competitive on the market. The maintenance system has to be cost-effective for the owner to enjoy the occupation of EAH and companionship of the animals. Therefore, EAH could be sustainable only where public attitudes are positive and appropriate premiums can be justified so that the systems remain viable. Product quality and origin should give added value to such production. Research and development should support such advancements and the adoption of new reasonable production systems serving animal welfare.*

(Keywords: extensive, intensive, animal husbandry, model, farming)

### **INTRODUCTION**

Animal husbandry (AH), stockbreeding, or simply husbandry, is usually defined as the agricultural practice of breeding and raising livestock. It has been practiced for thousands of years, since the first domestication of sheep around 9000 B.C. Extensive animal husbandry (EAH) has its origin in pastoralism, which refers to methods of conducting herds to natural pasturage, and therefore to systems where livestock raising is practiced in an extensive manner with little input and without the cultivation of fodder crops. It involves extensive exploitation of natural grazing lands entailing moves over varying distances. Actually, pastoralism has formed an intermediate stage between hunting and farming. Because of that, Alvard and Kuznar (2001) name AH „prey conservation“. Later on, integration of cultivation has developed pastoralism into agropastoralism. Selective breeding for the improvement of livestock was already practiced in Roman times. However, continuing systematic development and improvement of domestic livestock breeds was encouraged just in 18<sup>th</sup> century by Robert Bakewell, an English agriculturalist, who introduced modern

stockbreeding methods that transformed the quality of cattle, horses and sheep. Subsequently, integration of science into AH has introduced a new term – animal science. It should include controlled cultivation, management, and production of domestic animals, including improvement of the qualities considered desirable by humans by means of breeding.

However, after decades of improvements, which qualities do we consider desirable? And are they considered desirable by animals, as well? And how are we going to achieve them? Do we really care enough about ecology and welfare of domestic animals, or “breeding for money” has taken the main place? Are we capable to avoid abuse and ethically unacceptable conditions in so-called modern animal husbandry? And how could we motivate farmers to provide animals with things that they were faced to in their past, in the time of their wildlife? Finally, is the intensive animal husbandry (IAH) still modern and how big are the chances for the EAH to compete with it?

### **TRENDS IN ANIMAL HUSBANDRIES MODELS**

Generally, we have been used to distinguish the two models of AH; extensive animal husbandry (EAH), which is related to lower number of animals, extensive conditions and family farming and intensive animal husbandry (IAH), related to higher number of animals with higher productivity and industrial farming that supports high productivity.

The decision on which AH model to implement is much depending of land or climate that each country has got. For instance, in Ireland, United Kingdom, or France, agricultural area is much suitable for the production of forage, which is the precondition for the EAH. Generally, lowlands are more suited to crop production, while hilly areas often have high proportion of natural grassland. Furthermore, grazing is more usual in the west of Europe, while feeding based on cereals is found in the more southerly parts of Europe and this also enables fattening and finishing. This has caused differences in models of AH between north and south, so that farming systems that put the greatest emphasis on productivity are concentrated in the north and centre of Europe. They are characterised by high productivity of land, capital and labour, as well as wide variety of farming systems. In these parts of Europe, grassland farming has been for a long time extensive providing grazing for up to two cows per hectare. However, today even most of these farms are using more intensive methods through cooperation, usage of high producing breeds, artificial insemination, concentrates, artificial fertilizers etc. Farmer-producers that use crop production to provide most of the forage needed for livestock production are becoming relatively labour-intensive with over two cows per hectare. Mixed farms can be distinguished, as well, as the farms that are giving roughly equal importance to livestock and crop production, but productivity varies according to the region.

Due to the low amount of grasslands in south Europe, those countries can hardly compete in beef production to the north and central European countries and, therefore, they often have to import meat from those countries in order to satisfy their consumption habits. Other option is to fatten their animals on cereals, which is, for instance, situation in Italy, where forage area is relatively restricted and there are not so many grasslands, which has resulted with increased number of IAHs. Intensification of farming in lowland areas of southern Europe was carried out by introducing high productivity, reduction in numbers of dairy herds and shifting to cattle breeds that can be used for fast fattening and finishing. Feeding commercial concentrates which make animals grow faster and produce more has taken place of natural diet appropriate to the requirements of the animal. These large farms usually work under contract to major agri-food firms, cooperatives or private companies. This tends to be a rule in the EU as the total main forage area has receded falling from 55

million hectares in Europe (EU-15) in 1988 to 41 million hectares in 2001, i.e. a drop of 20% in thirteen years (*BioVision*, 2010). This trend is not welcome as EAH plays an important role in the protection of nature, mainly through preventing proliferation of invasive plants and subsequent gradual revert to woodland and reducing risks of fire. EAH, thus, helps to preserve biological balance. Furthermore, it is frequently the only way to maintain economic activity and population levels in some rural areas, especially in mountain areas. However, despite of ecological benefits of EAH, these systems have to be controlled concerning overgrazing that can cause erosion which is difficult to re-cultivate. For instance, the agro-pastoral farming zones in China have been seriously damaged due to overgrazing and reclamation disturbances, thus endangering the development of farming and AH, as well as threatening national ecological security (*Ding et al.*, 2007). Semi-natural grasslands resulting from traditional land use practices (mowing and grazing) are severely endangered throughout Europe, as well, due to the intensification of agriculture (*Saarinen and Jantunen*, 2005). Therefore, a combination of shed feeding and grazing in a fenced area may be an ideal combination of high productivity and animal friendly husbandry.

### **THE CROSS-FIRE BETWEEN ECONOMIC, HEALTH AND WELFARE CONSTRAINTS**

Through the years, the IAH has been called modern animal husbandry (MAH). In last 60 years, world number of inhabitants has increased from 2.5 to 6.8 milliards. However, agricultural lands are today covering nearly the same surface as before. Food industry has significantly started affecting eco-system and it is more and more clear that we cannot only produce. It has become more important how we produce, as well as how to produce food with economical usage of raw materials and energy with respect to the environment. Nowadays, we face the many facts that prove the IAH is not so modern anymore and that we have to search back for certain elements from the EAH in order to reactivate them in cost-efficient way. It has to be taken in consideration while integrating those elements into the IAH that high producing breeds require controlled living conditions that are hardly achievable in the EAH. Therefore, breeding strategy in such cases should be directed in strengthening disease resistance, often by sacrificing productivity of the animal. Benefits could be found in multifunctional usage of animal, for instance meat and eggs, and longer life with continuous production.

MAH managers are expected to confine populations of animals in compliance with economic, health and welfare constraints, while at the same time facing the strict scrutiny of public opinion that demands effective intervention and control strategies for production diseases. However, the cross-fire between economic, health and welfare constraints and the need for effective interventions in complex systems that cannot be resolved by human intuition alone represents the paradox of modern animal health management (*Döpfer and Morlán*, 2008). The IAH systems, however, are not necessarily balanced ecosystems and may develop dynamics that may not be predicted by intuition alone (*Edelstein-Keshet*, 1988). The scientific problems related to the well-being of animals reduced a complicated social critique of industrial systems for raising livestock to a matter of adapting animals to the living conditions imposed by these systems (*Porcher*, 2003). This swept out of view questions about the working conditions for farmers and wage-earners. However, people and animals tend to share living conditions in these systems that often cause suffering. Given the intensified pace of work, as people tend an ever larger number of animals, and the mounting pressure on both people and livestock, affects are repressed, and communication breaks down.

Relations to one's self and to others are altered, and the relation to death is "pathologized" at the workplace - thus providing further evidence of a failed relation to life and to others in AH. The public opinion is sceptical with regard to modern IAH (Fuchs, 2001). Society asks much more for production systems with high standards in animal welfare with grazing, straw beds and outdoor climate housing.

The competitiveness of IAH versus higher standards in animal welfare and the economic differences is becoming more present today. According to Brambell (1967) the provision of pig welfare on farms is dependent on the well-being and motivation of a farmer. Five freedoms: freedom from hunger and thirst; freedom from discomfort; freedom from pain, injury or disease; freedom to express normal behaviour; and freedom from fear and distress, are becoming more stressed today than ever (FAWC, 2007). Different production systems reveal different welfare problems. For instance, according to the results of investigation carried out in Croatia (Wellbrock *et al.*, 2009), from the perspective of resources pig welfare is better ensured on farm enterprises but from the perspective of animal-based welfare indicators no difference was found between the different pig production systems. The research findings indicate that modernisation of production systems is not likely to significantly improve pig welfare because a number of outlined welfare problems are related to lacking knowledge and education and cannot be overcome by modernising farming premises. Furthermore, allowing animals to express natural behaviour does not guarantee their welfare. In fact, natural behaviour can sometimes reduce welfare. According to Matthews (1996), there is a need to develop integrated measures of welfare that take account of longevity, disease, reproductive success, nutritional and thermal challenges, pain and behavioural freedom. Only with the help of such measures can acceptable practices be defended, unacceptable systems phased out and the consumer assured that EAH is animal friendly. Intensification of farming has put the question of how to keep animals healthy and new diseases have been occurring frequently (Antunović, 2000). In old European member states the intensification of pig production has led to ethical concerns regarding the welfare of pigs on farms (Veissier *et al.*, 2008). In Croatia, the EAHs are not interested in improving pig welfare because they do not want to increase productivity, but fear for their existence. As Croatia is today counting 31.845 small producing units covering 75% of whole pig production, these results represent considerably important indicators of necessity to approach this population of farmers.

Preventive measures in order to keep animals healthy, rather than on curative methods are taking place in every god managed system, no matter if EAH or IAH. However, preventive and unauthorised usage of veterinary drugs has raised potential risk to human and animal health through bacterial resistance (Banović *et al.*, 2008). Due to potentially huge losses in cases of diseases, as well as higher susceptibility on diseases (lower disease resistance), IAH farmers have been practicing prophylactic usage of antibiotics for many years. On other side, EAH farmers have been used to buy the medicaments by themselves and treat the animals. Large worldwide surveillance studies report that resistance to nearly all classes of antimicrobials are increasing, as is the emergence of what have been termed pan-drug-resistant and extremely drug-resistant pathogens (Owens, 2008). This indicates necessary to sacrifice part of productivity, improve the living conditions and hygiene of animals, allow animals to practise their natural behaviour and avoid social stress in order to gain human and animal health. For instance, the main principle for veterinary treatment in the organic animal husbandry (OAH) is: get to know the causes of (or factors that favour) diseases in order to enhance the natural defence mechanisms of the animal. The principle of changing management practices in order to prevent disease outbreaks should be applied more especially in the

IAH, as well. Often, the problem of investments in such changes presents a problem to producers and governmental financial help in certain percentage is always welcome. Good example is governmental support for egg producers in Croatia in order to increase cage area per hen according to the EU rules (EC, 1999). Yet, little effort has been shown to implement the EU welfare directives on farms in Croatia (EC, 2006).

### **SMALLHOLDINGS AS A WIDESPREAD TRADITION IN SOUTH AND EAST EUROPE**

Contrary to the central and west Europe, where many EAHs have been intensified, there is less emphasis on productivity in EAH of southern Europe, characterised by low productivity of capital and labour, variable productivity of land, wide variety of farming systems and location in areas where GDP is below the European average. In south upland areas of Europe, EAH with low land productivity tend to be the rule. This type of traditional farming is hardly industrialized at all. Smallholdings are a wide-spread tradition in new South-Eastern and Eastern European member and candidate countries but often neglected in the European alignment process (Thurston, 2008). They serve, however, a social safety net function for many rural inhabitants in new European and EU candidate countries with socialist and communist histories (Vira and Narnicka, 2003). An example could be found in Romania, where a dramatic decline in the economic situation of the rural population in mountainous regions has been determined, resulting in far reaching ecological consequences (Pfeuffer and Sambraus, 2006). Here, consideration was given as to which steps should be taken in order to improve the economic situation of the population and therefore preventing further ecological changes. Solutions were sought as to how AH could be improved quantitatively and qualitatively. However, it would appear that improvements could only be realised if the broader situation changes. This model can have cultural or religious significance, but often cannot contribute much in increasing of GDP from agriculture or export of a country. What is more, we often find such EAH producers to be hardly competitive on the market, which can result with giving up the production.

The Croatian Agriculture and Rural Development Plan (MAFWM, 2006) has predicted family AHs to become leading producers in agriculture. Croatian agriculture is participating in GDP by 10%, or 13.5% of total number of employed. Small-scale farming, especially for own consumption, is deeply rooted in Croatian rural culture. Croatian officials aim to create favourable conditions for social-economic development in its most backward regions and to maintain traditional features of the Croatian EAH. However, lacks of processing industry, as well as high import of food have resulted in slow development of AH and often giving up the business by the farmers. It has been estimated that 2–4 AHs are giving up the business daily in Croatia. While milk production in 2009 has increased for 20 million litres compared to 2008, number of producers decreased for 4,000. Compared to 2002, number of milk producers in 2009 in Croatia decreased from 65,000 to 23,690. Number of swine decreased from 200,000 in 2005 to 132,000 in 2009. On the other side, many big producers have decided to base their management on import rather than on investments in own production, clusters and marketing, which is ruining competitiveness of small farmers. Import is much disturbing swine market in Croatia as for instance only from Chile 1,400 tons of frozen pork has been imported in Croatia in three months. Annually, Croatia is importing food worth 15 milliards Euro, which has significantly reduced marketing possibilities for Croatian farmers. Often, food processing industry (like meat products etc.) that would bring extra value to the primary production is missing.

The Croatian AHs received from the Government 180 million Euros of subsidies in 2009 and it has been planned to direct another 95 million Euros in 2010. This decision to decrease annual amount of subsidies for 40% has caused farmers' rebellion with obstruction of roads with tractors etc. Farmers are mostly used to see subsidies as a social category, rather than development measure. From the total number of 190,000 registered farmers in Croatia, 90,000 are receiving subsidies, but in the same time 70% of them do not have any agricultural education background. For comparation, in Germany that has 17 million ha of agriculture land only 140,000 of farmers are receiving subsidies. Now we have situation in Croatia that non-educated farmers often cannot produce quantity and quality to become competitive and cannot give back credits to the banks, so they rely on subsidies. It has been shown that a lack of communication efforts by public administration officers results in a lack of knowledge amongst EAHs concerning EU accession changes and production standards (*Wellbrock et al.*, 2010). Furthermore, many properties are not registered by the Government. Currently, from the total number of 800,000 ha of agriculture land, 545,000 ha are governmental, but in the same time 360,000 ha of land are not registered and so unknown if and who is working on these properties. Without legalisation of these properties, they would not be able to go to the market after the EU accession of Croatia. Subsidies from EU for Croatian agriculture should start in 2012. The politics of EU subsidies is different than current politics of subsidies in Croatia. It is less purposed for direct payments and more purposed for rural development, respect to ecological issues etc. Consequently, in order to get subsidies in the future, the AH will have to register their properties and go through the education program.

Land properties and AHs in Croatia are relatively small compared to the EU average. Currently, 135,000 AHs are active in Croatia. However, only 5,000 of Croatian AHs have 10 to 20 ha of land and only 1,000 AHs have more than 20 ha of land. All the others are small producers. Around 50% of AHs that produce milk have up to 4 cows and 98% of AHs that produce pork have up to 10 swine. From the total number of Croatian pig farms, 75% of production has been organised in small production units (1–5 sows) accounting for within the activities of the mixed family holdings. Croatian pig production systems are thus comparatively smaller than in older EU member states where 67% of all family farms own between 100 and 200 breeding sows (*Antunovic et al.*, 2004a). Not many farms in Croatia are specialised production units with up-to-date technologies and complying with the EU standards. To choose whether farms should modernise their production or terminate their production, the Croatian Government has distinguished pig farms into commercial and non-commercial farms. The distinguishing criteria for being considered a commercial or non-commercial farm is the amount of pigs produced. Commercial production units have to be registered and can ask for financial support from modernisation and production funds, capital investments or rural development funds. Non-commercial production units, however, do not have to be registered as producers and can only ask for financial support through rural development funds. So, the question is how the family AHs with such small land properties per AH, that Croatian Government is in favour (*MAFWM*, 2006), and with such a big food import is going to compete on the EU market where there is tendency to form properties of 80 ha.

## **POSSIBILITIES OF UPRISING EXTENSIVE ANIMAL HUSBANDRY IN THE FUTURE**

To circumvent welfare impairments for smallholders in Croatia and other new European member and candidate countries, it appears necessary for the EU to recognise

smallholding as a type of farming in legislation. This way, one can argue, smallholders are more likely to be addressed with financial aid and education measures in the future. It is therefore necessary to invest in further research regarding the ways in which smallholders can be supported in the future (Wellbrock *et al.*, 2010).

The EAHs show tendencies for economic disadvantages due to higher labour costs, additional handling of straw and especially if performance declines for the production systems with higher animal standards. It requires more land, dung is spread on the pastures and it is often resulting with lower productivity. So, it is crucial to select the right kind and number of farm animals that he can support with land, labour and health care. In warmer seasons, grazing can save labour costs and enable animals better movement and exercise, resulting with better health condition. There are low cost production systems under development. One example is outdoor climate housing for pork production which seems to be economically competitive (Antunović *et al.*, 2004b). The benefit of EAH could be found in by-products such as straw, biomass from field margins or kitchen wastes that can be used as cheap and easily available fodder, as well as in the dung that should be returned to the fields in the most efficient way in order to increase the fertility of the soil. Animal products such as milk, eggs, and meat can both be used for home consumption in the family as well as for selling, thus generating additional income for the farmer.

The results of such attitude are standards given through the Good Agricultural Praxis (GAP) that represent a collection of principles to apply for on-farm production and post-production processes, resulting in safe and healthy food and non-food agricultural products, while taking into account economical, social and environmental sustainability. They provide the opportunity to assess and decide on which farming practices to follow at each step in the production process. For each agricultural production system, they aim at allowing a comprehensive management strategy, providing for the capability for tactical adjustments in response to changes. The implementation of such a management strategy requires knowing, understanding, planning, measuring, monitoring, and record-keeping at each step of the production process. Adoption of GAP may result in higher production, transformation and marketing costs, hence finally higher costs for the consumer. AH advisors, a profession which supplies advice to animal owners on matters of husbandry, are becoming important for EAH owners who are not supported with multidisciplinary management as IAH owners.

Nowadays, organic animal husbandry (OAH) is increasingly being seen as one sustainable option to farmers, among the alternatives to conventional input of IAH. Term OAH means integration of AH into crop producing farms with a central focus on the welfare and health of the animals and it is often being wrongly called EAH, which is different in meaning of fewer demands on environmental conditions. There is a range of standards for OAH regulating the management, shedding, feeding, veterinary treatment, breeding, purchase, transport, and slaughter of farm animals in detail (IFOAM, 2005). OAH offers especially good conditions to farmers with high activity in marketing. However, it requires an intensive education of farmers in agrarian ecology (Schumacher, 1998) and land support. Its development is different and often slow, depending on each country. Organic producing in Croatia started in 2002 with two husbandries registered on 54 ha of land, while today there are 817 husbandries registered on 14,193 ha of land. However, with 1.3% of land with organic husbandry, Croatia is still far away from the EU average (4.1%). For instance, Italy or Spain have more than 1 million ha of land with organic husbandry and Austria has 15% of such land. Currently, 14 millions of

husbandries on 7.2 million ha in EU are organic. Farm tourism could be recognised as a model of choice, as well, which makes possible to combine farming and the protection of the environment with the development of quality products. It has expanded in last decades and has been recognised as a model which preserves biodiversity.

## CONCLUSIONS

Extensive animal husbandry is becoming modern again, especially concerning preserving biodiversity, rural development, protection of natural heritage, traditions and animal welfare. However, it depends much on land support, land-use management, climate, economical development of each country, education and motivation of farmers and possibilities to be competitive on the market. It could be sustainable only where public attitudes are positive and appropriate premiums can be justified so that the systems remain viable. The maintenance system has to be cost-effective for the owner to enjoy the occupation of AH and companionship of the animals. Product quality and origin should give added value to such production. Research and development should support such advancements and the adoption of new reasonable production systems serving animal welfare.

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## **Effects of production potential, system of production on environmental footprint in different animal species**

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### **ABSTRACT**

*Growing world population and increasing purchasing power increases the world consumption of animal products. To consider aspects of more efficient utilization of basic resources as water, feed etc. requires more attention, combined with the growing concern regarding, the environmental footprint, per unit product and its interrelationship with the production potential of the animal populations, and systems of production. In latter context research results are reviewed. Poultry, pig, sheep dairy cattle and beef production examples are presented, showing clearly that more productive populations and systems are characterized by significantly reduced environmental footprint per unit product compared to less efficient ones. Water utilization as an example was improved due to genetic improvement in broilers and turkeys by 250–300% in breast fillet meat production in the last 30 years. Dairy production system developments in the USA between 1944 and 2007 reduced the environmental footprint, and reduced the necessary feed production area dramatically among species the more prolific ones have a competitive edge if production is based on feeds produced by the arable agricultural sector.*

(Keywords: environmental footprint, unit product, level of production, systems of production, poultry, pig, cattle)

### **INTRODUCTION**

Growing population numbers and increasing per capita incomes in many parts of the World impose an ever growing demand for human food and animal feed supply. The bio energy production is a new serious competitor. The natural basic resources, land available and soil quality is diminishing and deteriorating in several areas of the World. Ground water levels shrink, the border conditions for irrigation worsen both in several developed (USA, Australia etc.) and in developing countries (China, India etc.) (Diamond, 2007). Marine – and to a lesser extent – freshwater living fish populations have been dangerously reduced due to extreme overfishing. Fish represents a vital and is the sole animal protein source for more than 1.2 billion people in the developing world, and constitutes a valuable part of the healthy human diet in developed countries (Diamond, 2007). Marine and freshwater fish production waits for a real new revolution: development of novel artificial breeding systems, establishment of large protected marine and freshwater ecosystems, innovative fishing technologies, strictly and efficiently enhancing a much more sustainable type of approach regarding the maintenance of fish populations both quantitatively and qualitatively worldwide (Horn, 2007). Climate change will probably negatively influence plant agriculture both

quantitatively and qualitatively. Risk factors will increase as a large number of reliable publications call our attention to the problem.

Growing per capita income linearly increases consumption of foodstuffs of animal origin, most significantly meat. Below 1500 US \$ annual family income the food is of plant origin. Above this income level people begin to consume also food items of animal origin (Roppa, 2007). The switch from a vegetarian diet to animal products requires a 4 to 12 fold plant biomass production – depending on the type of animal product – due to transformation losses. Animal agriculture faces great changes and challenges.

### **Water utilization and production potential**

In the context of the global climate change, it seems more and more important to consider water utilization efficiency per unit product in animal agriculture. In Kaposvár several evaluations were conducted in the last years to compare water utilization efficiency with numerous genotypes characterized by significantly different performance levels. In chickens the 1978-, 1998- and 2008-type typical broilers were compared regarding water utilization to produce 1 kg of breast fillet meat.

The 1978-type broiler needed 40 l drinking water, and 20,000 l of precipitation water to produce the feed for 1 kg breast fillet meat production. Due to genetic change in 30 years in 2008, broilers needed only 14 l drinking water and 7000 l precipitation water for the same realized production. Roughly at present only one third of water is necessary to produce the same amount of breast meat fillet as 30 years ago (Horn, 2005; 2008). Comparing Turkey strains representing 1967 and 1999 types, and reared under identical environmental and feeding conditions (Herendy *et al.*, 2004) it was found that 1967 type bronze turkeys needed 50.6 drinking water, and 25,300 l of precipitation water (feed production) to produce 1 kg of breast fillet meat, whereas the 1999 type turkey needed only 21 l drinking water, and 20,500 l of precipitation water for the same product (Horn, 2007).

Calculations showed that water utilization of dairy cows per unit milk production diminished by 1.6 l regarding drinking water, and is reduced by 427 l considering the water (precipitation) quantity needed for feed production if milk production rises from 4000 to 12000 kg/year cow. Increased production from 4000 to 8000 litres of milk saves 1.1 l drinking water and 312 litres of precipitation water for 1 kg milk (FCM) produced (Babinszky and Horn, 2005), cit. Horn (2005, 2007)

Improvement in genetic potential significantly improves water utilization efficiency per unit product. In monogastrics (meat type poultry) the correlation between levels of performance is very close to linearity with water usage efficiency. In milk production this relationship is different, the higher the level of the cows' genetic potential, further gains yield smaller and smaller improvements in water efficiency to produce unit amount of milk.

### **The environmental footprint and animal production**

It is of great importance in the future to consider the differences existing between species, genotypes within species and systems of production related the environmental footprint per unit of product destined for consumption.

The large scale evaluations published by Williams *et al.* (2006), show that between different livestock sectors very large differences exist in inputs and several components deteriorating the quality of the environment in a complex manner (glass house effect, eutrophycation potential, pesticides use, land use) determining the environmental footprint (*Table 1*).

**Table 1**

**The main burdens on environment and resources used in animal production per tonne of meat, per tonne of eggs (20,000) per tonne of milk dry matter (10 m<sup>3</sup> milk)**

Impacts and resources	Poultry meat	Eggs	Pork meat	Beef	Milk	Sheep meat
Primary energy used, GJ	12	14	17	28	25	23
Global warming potential 100 year time scale, CO <sub>2</sub> t	4.6	5.5	6.4	16	10.6	17
Eutrophycation potential, PO <sub>4</sub> kg	49	77	100	158	64	200
Acidification potential, SO <sub>2</sub> kg	173	306	394	471	163	380
Pesticides used, kg/ha	7.7	7.7	8.8	7.1	3.5	3.0
Land use, ha	0.64	0.67	0.74	2.33	1.20	1.40

Source: *Williams et al.*, 2006

Broiler chicken, egg and pork production have a smaller environmental footprint compared to other production sectors. CO<sub>2</sub> output is an important contributor to global warming (as declared by the majority of experts, although by far not all).

In *Table 2* the CO<sub>2</sub> production of fattening pigs as affected by weight and growth potential are tabulated (*Jentsch et al.*, 2009).

**Table 2**

**CO<sub>2</sub> production of fattening pigs as affected by live weight and growth potential**

Bodyweight (kg)	BW gain (g)	CO <sub>2</sub> production (kg/kg BWG)
40	500	134
	700	1.26
60	400	1.85
	600	1.58
	800	1.46
80	400	2.11
	600	1.82
	800	1.67
100	500	2.11
	700	1.87
120	500	2.26
	700	2.02

Source: *Jentsch et al.*, 2009

In pigs during the fattening period an increase in daily weight gain by 200 g, decreases CO<sub>2</sub> emission by 10–15% per kg gain.

Very similar tendencies were published as for pigs for fattening bulls in the weight classes 200, 300, 400 and 500 kg. The CO<sub>2</sub> output per kg bodyweight gain was reduced if daily gain improved 400 g in the various weight classes by 17.3, 9.4, 8.6 and 8.5% respectively (*Jentsch et al.*, 2009).

Of special interest and importance is a publication of *Capper et al.* (2009) comparing the complex environmental impact of dairy production of the USA characteristic for 1944 and 2007 (*Table 3, 4*). The objective of that study was to compare the environmental impact of modern (2007) US dairy production with historical production practices as exemplified by the US dairy system in 1944. “The summary of this paper clearly demonstrates the huge impact of both genetic and managemental improvements on overall efficiency and reducing environmental pressure in milk production.” A common perception is that pasture based, low-input dairy systems characteristic of the 1940s were more conducive to environmental stewardship than modern milk production systems. A deterministic model based on the metabolism and nutrient requirements of the dairy herd was used to estimate resource inputs and waste outputs per billion kg of milk. Both the modern and historical production systems were modelled using characteristic management practices, herd population dynamics, and production data from US dairy farms. Modern dairy practices require considerably fewer resources than dairying in 1944 with 21% of animals, 23% of feedstuffs, 35% of the water, and only 10% of the land required to produce the same 1 billion kg of milk. Waste outputs were similarly reduced, with modern dairy systems producing 24% of the manure, 43% of CH<sub>4</sub>, and 56% of N<sub>2</sub>O per billion kg of milk compared with equivalent milk from historical dairying. The carbon footprint per billion kilograms of milk produced in 2007 was 37% of equivalent milk production in 1944. To fulfil the increasing requirements of the US population for dairy products, it is essential to adopt management practices and technologies that improve productive efficiency, allowing milk production to be increased while reducing resource use and mitigating environmental impact.”

**Table 3****Characteristics of the 1944 and 2007 dairy production systems**

Variable	1944	2007
Breed	54% Jersey/Guernsey/Ayrshire (small) 46% Holstein/Brown Swiss (large)	90% Holstein
Milk yield per cow, kg/yr	2.074	9.193
Milk fat content, %	4.20 (small breed) 3.60 (large breed)	3.69
Milk protein content, %	3.50 (small breed) 3.20 (large breed)	3.05
Heifer: cow ratio	0.89	0.83
Heifer growth rate, kg/d	0.42 (small breed) 0.59 (large breed)	0.68
Age at first calving, mo	27.0	25.5
Breeding method	100% natural service	70% AI, 30% natural service
Bull: cow ratio	1:25	0:83
Principal forage sources	Pasture, hay	Corn silage, alfalfa silage
Diet type	Forage + concentrate	Total mixed rations

Source: *Capper et al.*, 2009

**Table 4****Comparison of resource inputs, waste output, and environmental impact of dairy production systems in 1944 and 2007**

Variable	1944	2007
Milk produced, billion kg	53.1	84.2
Resources/waste per billion kg milk produced		
<i>Animals, n</i>		
Lactating cows, $\times 10^3$	414.8	93.6
Dry cows, $\times 10^3$	67.4	15.2
Heifers, $\times 10^3$	429.2	90.3
Mature bulls, $\times 10^3$	19.29	1.31
Adolescent bulls, $\times 10^3$	17.17	1.08
Total population, $\times 10^3$	948	202
<i>Nutrition resources</i>		
Maintenance energy requirement <sup>1</sup> , MJ $\times 10^9$	16.66	3.87
Maintenance protein requirement, kg $\times 10^6$	165.4	48.4
Feedstuffs, kg of fresh weight $\times 10^9$	8.26	1.88
Land, ha $\times 10^3$	1.705	162
Water, L $\times 10^9$	10.76	3.79
<i>Waste output</i>		
Nitrogen excretion, kg $\times 10^6$	17.47	7.91
Phosphorus excretion, kg $\times 10^6$	11.21	3.31
Manure, fresh weight, kg $\times 10^9$	7.86	1.91
Gas emission		
Methane <sup>2</sup> , kg $\times 10^6$	61.8	26.8
Nitrous oxide <sup>3</sup> , kg $\times 10^3$	412	230
Carbon footprint <sup>4</sup> , kg of CO <sub>2</sub> $\times 10^9$	3.66	1.35

Source: Capper *et al.*, 2009

<sup>1</sup>Refers to nutrient required for maintenance (all animals), pregnancy (dry cows), and growth (heifers and adolescent bulls); <sup>2</sup>Includes CH<sub>4</sub> emissions from enteric fermentation and manure; <sup>3</sup>Includes N<sub>2</sub>O emissions from manure (both years) and from inorganic fertilizer application (2007 only); <sup>4</sup>Includes CO<sub>2</sub> emissions from animals, plus CO<sub>2</sub> equivalents from CH<sub>4</sub> and N<sub>2</sub>O.

Based on Capper *et al.* (2009) data if the 1944 type typical milk production system would be applied at present, 143 million ha of land would be needed to supply the US population with 84 billion kg of milk. This would require 1/3 of the USA total agricultural area. The present system needs only 13.6 million ha-s (Horn, 2009).

To supply mankind with adequate animal products both quantitatively and qualitatively it is indispensable to utilize genetically further improved populations and complex managemental systems. This will be more important than ever before.

## CONCLUSIONS

The manifold new challenges facing animal agriculture forces us to reevaluate production efficiency in a more and more complex manner. The pressure to utilize all natural (and

also human) resources more efficiently (water, feeds available, energy, land etc.) to meet growing demands both quantitatively and qualitatively inclusive food safety (all three are closely interrelated) we must be aware of the fact that in most cases to utilize highly productive genetic stocks, and matching production environment is inevitable. Efficient complex systems of production tend to have a reduced environmental footprint per unit animal product produced in all main species (poultry, pigs, cattle etc).

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## Livestock systems and environment on the Alps

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### **ABSTRACT**

*Extensive livestock systems in mountain areas have a relevant ecological rule. Traditional farms, based on use of local forages and pastures contribute to maintain environmental quality and biodiversity of mountain ecosystems. Mountain agriculture experimented a dramatic decline in the last years, a phenomenon mostly driven by socio-economic factors such as immigration into lowland where new economic opportunities are offered to rural people. The abandoning of traditional extensive farming has been associated with land use changes and loss of biodiversity. This paper analyse the evolution of livestock farms in the Alps, focusing on environmental sustainability of production systems.*

(Keywords: livestock systems, mountain, environment)

### **INTRODUCTION**

In recent decades European agriculture has experienced radical changes, with a decrease in farm numbers and the abandoning of traditional extensive farming in favour of highly mechanised and intensive production practices (Caraveli, 2000; Höchtl *et al.*, 2005; Strijcker, 2005). This process has been particularly dramatic for the traditional extensive livestock farms of the Alpine region (Caraveli, 2000; MacDonald *et al.*, 2000; Bonsembiante and Cozzi, 2005; Lasanta *et al.*, 2006).

It is generally accepted that extensive farming practices increase environmental quality and biodiversity of agro-ecosystems as compared to intensive ones (Hoogeveen *et al.*, 2002). More specifically, the abandoning of traditional farming in mountain areas has been associated with extensive reforestation (Garcia-Ruiz *et al.*, 1996; Bebi and Baur, 2002; Gellrich *et al.*, 2007), soil degradation, loss of biodiversity (Conti and Fagarazzi, 2005), and a decrease in landscape quality and attractiveness (Giupponi *et al.*, 2006).

The aim of this paper is to analyze the evolution of livestock systems in mountain regions, taking the Alps as study area, and to investigate the relationships between animal husbandry and environmental features.

### **Livestock systems in European mountain areas**

Animal husbandry is largely diversify across European mountain areas. Geographic and climatic traits represent limits for feedstuff production, traditionally based on forages and pasture. For centuries, cattle and small ruminants able to optimize these kind of resources were reared in extensive or semi-extensive systems (Cozzi and Bizzotto, 2004). A schematic classification of livestock systems is showed on *Table 1*. On the Alps, cattle husbandry is historically based on small herds of local dual purpose breeds for milk and meat production, housed in closed barns located in the valley during winter and moved to alpine summer pastures from June to September. Local dual purpose breeds well

adapted to mountain environment were widespread in the alpine regions, such as Simmenthal, Brown Swiss, Grey Alpine, Valdostana and Rendena in Italy, Abondance and Tarentaise in French and Herens in Switzerland.

**Table 1**

**Classification of livestock systems on Alps**

Livestock system	Management	Feeding	Reproduction	Products
Dairy cattle (or goat) without summer pastures	Free or tie stalls (free for goats)	Forages and on farm pastures	All year long	Milk and calves
Dairy cattle (or goats) with summer pastures	- Winter: Free or tie stalls - Summer: moved to alpine pastures	- Winter: forages and concentrates supplementation - Summer: pastures and concentrates supplementation	Seasonal or all year long	- Winter: Milk and calves (or kids) - Summer: milk or cheeses
Transhumance : sheep	- Winter: lowland, stalls; - Spring-summer: alpine pastures	Pastures with few supplementary feeding	Seasonal	Lambs (in some cases cheeses and wool)
Suckling cows	- Winter: stalls - Spring-summer: pastures	Forages and pastures	Seasonal	Calves

Modify from *Bovolenta et al.* (2008).

Milk were traditionally processed on-farm or in small cooperative cheese factories, and a large variety of high quality cheeses are still strictly connected with mountain dairy systems. Small ruminants are also reared to produce meat, milk and wool. Sheep and goats are able to use the less favourable pastures, where dairy cows are excluded. Traditionally small ruminants are reared in large flocks with low grazing pressure. An example of sustainable productive livestock farming is represented by dairy small ruminants for production of local cheese still present in some regions of Alps (e.g. Piemonte in Western Italian Alps, *Battaglini*, 2007). The most outstanding feature of sheep farming systems in some alpine regions is the vitality of the traditional transhumance based on the integrated use of alpine pastures and of hill and plain marginal foraging resources. This system was able to adapt to the changes in land uses and food consumption, and is still used in some area. An example is the husbandry of Bergamasca/ Biellese sheep in Lombardia for the production of heavy lamb (*Corti*, 2007).

These kind of extensive systems contribute to create and maintain the typical alpine ecosystems, with forest fragmented by open areas such as meadows and pastures (*Giupponi et al.*, 2006).

**Evolution of Livestock systems in the Alps**

In the last decades, the Alps experimented a general abandonment of traditional farms, with different regional trends. According to *Streifeneder et al.* (2005), the number of farms in the period between 1980 and 2000 decreased of 40% (*Table 2*). The highest percentage of ceased farms occurred in the most decentralised areas of the European Alps, where farm holdings, generally small and unprofitable, were abandoned (*Giupponi*

*et al.*, 2006; *Tasser et al.*, 2007). In the same context, in regions that are to some extent disadvantaged in relation to the natural-site conditions, such as *Südtiroler Berggebiet* and *Innsbruck Land* in Austria, as much as 37% of land has been dismissed. Similarly, in Carnia region, Italy, nearly 67% of the formerly agriculturally used areas have been abandoned too (*Tasser et al.*, 2007). In Austria and Germany changes were rather modest, whereas they were very strong in Italy, France and Slovenia. In particular the smallest farms ceased, with a tendency to increase the number of animals per farm. This process have led to choose more specialized breeds, such as Holstein Friesians or Brown-Swiss whose are common on the more intensive farms; small regional dual purpose breeds are maintained only in the small traditional herds.

Considering the use of permanent grassland, at the alpine border regions, in Germany, Slovenia and eastern Austria, husbandry is characterised by an high intensity (*Table 2*). On the opposite there are extremely extensive used regions in France and Italy. In general, livestock density does not increase due to additional livestock but due to decreasing areas of permanent grassland (*Streifeneder et al.*, 2005). In many alpine summer pastures stocking rates are managed at sub-optimal levels and therefore are only partially constrained by pasture productivity (*Mrad et al.*, 2009).

The evolution of animal husbandry shows different trend at regional level. In the same country is possible to have marginal areas with farm abandonment and other zones characterized by maintenance of sustainable livestock systems. An example of this situation is given by Eastern Italian Alps. The total surface covered by the three bordering provinces of Bolzano, Trento and Belluno is about 17,000 km<sup>2</sup>, and is classify as completely mountain area. In table 3 the changes of livestock indicators between 1980 and 2000 are reported (data from official censuses *ISTAT*, 1982 and 2002).

The province of Bolzano experimented a mild loss of dairy farms, with an increase of dairy cows and as a consequence an increase of herd size. The distinctive trait of Bolzano dairy sector is the maintenance of a large number of small familiar farms. The increase of small ruminants was higher, in particular the number of goats in 2000 was doubled with respect the 1980. The maintenance of sustainable farms have permitted to maintain grassland and pastures surface. The situation is different in Trento and Belluno. Both the provinces experienced a dramatic abandonment of small dairy farms, with a percentage of ceased farms of about 80% in only 20 years. In 2000 there were less farms with a higher herd size (11.2 cows/farm in Belluno and 16.8 in Trento, almost the double with respect to Bolzano). For sheep and goats a large percentage of farms closed, with a consequent increasing of herd size in the active ones.

The most evident consequence on landscape is the loss of open areas, which was moderate in Trento but relevant in Belluno (-8 and -25%, respectively). The abandonment of extensive farms based on marginal grassland caused a progressive encroachment of meadows and pastures, followed by reforestation. Also in the territory of these two provinces there are different situation. In the north of Belluno the farm abandonment and the consequent loss of open areas is strongest than in the south; in fact the north is characterized by higher elevation and in the south are concentrated the most economically viable farms and there is also the only industrial cheese factory of the province (*Giupponi et al.*, 2006).

In the Bolzano province there is a particular institution, the Tyrolean Closed Farm (*Geschlossener Hof*), which is a indivisible agricultural holding that has been passed down from father to first-born son for centuries. The maintenance of this tradition in an Autonomous Province totally mountainous with a strong touristic attractiveness permitted to avoid the livestock abandonment experimented by the other bordering areas.

Trento is also an Autonomous Province, and the policies aimed to sustain the agriculture and to promote the development of multifunctional farms helped to maintain a sustainable sector. At the opposite, Belluno is part of the Veneto region, where the main urban settlements and economic activities are located in the lowland. This different trends in similar environmental conditions should be explained by a range of factor, and this phenomenon should give relevant consequences on land-use changes. These aspects will be discussed in the next paragraphs.

**Table 2**

**Variation of farms and livestock units between 1980 and 2000**

Country	Agricultural farms, number			Livestock units (LU), total			LU/permanent grassland, ha		
	2000	1980	2000-1980 (%)	2000	1980	2000-1980 (%)	2000	1980	2000-1980 (%)
Austria	96,205	119,837	-19.7	1,076,656	1,210,981	-11.1	0.7	0.8	-8.3
Switzerland	26,562	41,363	-35.8	538,066	607,310	-11.4	2.0	2.2	-8.6
Germany	22,511	31,623	-28.8	661,064	705,028	-6.2	2.1	1.7	24.2
France	28,571	52,647	-45.7	384,604	563,752	-31.8	0.7	1.1	-34.6
Liechtenstein	199	494	-59.7	4,608	6,524	-29.4	1.8	2.2	-18.5
Italy	171,038	309,146	-44.7	642,546	900,283	-28.6	0.6	0.7	-14.9
Slovenia	23,149	53,089	-56.4	146,399	181,282	-19.2	1.4	1.2	15.2
Alps total	368,235	608,199	-39.5	3,453,943	4,175,160	-17.3	0.9	1.0	-8.9

Modify from *Streifeneder et al.*, 2005.

**Table 3**

**Evolution of livestock systems in Eastern Italian Alps**

Variable	Bolzano			Trento			Belluno		
	1980	2000	% variation	1980	2000	% variation	1980	2000	% variation
N of cattle farms	12,792	9,476	-26	6,435	1,678	-74	4,763	1,137	-76
N of cattle	139,708	144,196	3	61,446	45,050	-27	35,830	20,606	-42
N of dairy farms	12,317	8,565	-30	5,749	1,416	-75	4,317	807	-81
N of dairy cows	63,132	75,468	20	28,770	23,812	-17	16,097	9,043	-44
Dairy cows/farm	5,1	8,8	76	5,0	16,8	236	3,7	11,2	203
N of sheep farms	2,080	2,136	3	418	320	-23	740	342	-54
N of sheep	25,796	39,739	54	13,977	20,377	46	5,259	5,615	7
N of goat farms	1,397	1,725	23	820	429	-48	855	244	-71
N of goat	7,930	15,714	98	2,923	5,132	76	2,262	2,318	2
Grassland and pastures (ha)	230,163	240,153	4	120,316	110,196	-8	63,349	47,446	-25
Crops (ha)	7,542	3,780	-50	6,583	1,982	-70	4,892	5,232	7

**Drivers of changes in alpine livestock systems**

The rapid changes of European agriculture were determined by various factors, in particular socio-economic development and innovation in farm technology. At the same time, significant changes in land use were observed with large implications for the sustainability of grazing agro-ecosystems (*MacDonald et al.*, 2000). In the Alps, increasing economic pressure to maintain farm incomes has resulted in intensification of the more accessible (and productive) soils and in a partial abandonment and fragmentation of semi-natural habitats, characterized by soils with a low nutrient status

and farms with high labour requirements. The main cause of farm abandonment across the entire alpine region is the retirement of farm managers (*MacDonald et al.*, 2000). Potential successors and heirs are not interested in taking over the operation of a farm since the income thus generated is less than satisfactory and employment opportunities are more attractive in other sectors. Since potential successors move to regions with better employment opportunities, as already mentioned, regions with high migration rates often entail a decrease in the agricultural sector. In the eastern Italian Alps is this the case of the Belluno province, where in last decades the development of small industries (in particular for glasses production) and services connected to the tourism led to farms decline (*Giupponi et al.*, 2006). Regions with a sound regional-economic environment and, therefore, a relatively stable population trend on the other hand register lower rates of farm abandonment.

In terms of the general socio-economic context that surrounds ruminant livestock farming in Europe, the Common Agricultural Policy (CAP) plays a central role, resulting in a polarization of production areas and a loss of mixed farming. Although it has prevented some low-intensity systems with high biodiversity from being abandoned, it has also led many marginally economic areas to be forsaken (*MacDonald et al.*, 2000). The successive reforms of the CAP tried then to recognize the important role of farms in the sustainable management of territories: agri-environmental measures in 1992, rural development policy and farm territorial contracts in 1999, and payment based on environmental criteria including the reform of the CAP in 2003 (*Gibon*, 2005). CAP 1992 (expanded by the Agenda 2000) implemented compensation payments and extensification policies that have caused enlargement of herd sizes and great dependency on subsidies (*Veyset et al.*, 2005); production objectives declined and, to a large extent, farmers' gains were based on maximising the capture of premiums. The mid-term review of the CAP implied decoupling of subsidies from production and a progressive regression of economic support, which might result in changes in the observed trends as a consequence of new adaptation strategies of farmers. Furthermore at farm level the internal structure and economy influence the evolution of mountain agriculture. One fundamental aspect that explains the permanent disappearance of farming and subsequent abandonment of large pastoral areas in the Alps is the lack of continuity of agricultural households, directly related to the presence and number of descendants, but other aspects are also involved. One of the most relevant ones is the high opportunity cost of household labour, especially for the younger family members. These general factors, associated with other local specific situations, contributed to abandonment of mountain farming, which was for centuries the main occupation for people living on Alps.

### **Effects of changes in alpine livestock systems**

Environmental impact is one of the emerging problems of the livestock sector. In mountain areas the environmental issues related to evolution of livestock systems are land use changes and loss of biodiversity, as reported by many authors (*Table 4*). In a general review on agricultural abandonment in mountain areas, *MacDonald et al.* (2000) showed that the reduction of traditional farming practices (such as transhumance or hay meadow management), or land abandonment cause loss of biodiversity, loss of open pastures, reforestation, and also a loss of characteristic decentralised farm settlements. In fact, in mountain areas already dominated by high forest cover, increased woodlands caused by abandonment processes may not be desirable, leading to a biodiversity loss due to a diminished variety of habitats: when small patches of open meadows, woodlands and

cultivated fields shape landscape, then forest expansion might result in an increased landscape homogeneity and banalisation (Conti and Fagazzari, 2005).

In the Swiss Alps, *Gerllich et al.* (2007) evidenced that intensification is not convenient, because farm labour costs increased faster than incomes, leading to under-grazing and the cessation of tree and shrub clearance.

In the eastern Italian Alps, different studies demonstrated that the decline of traditional extensive livestock systems caused a loss of open areas, with a process of reforestation, simplification of the landscape and loss of biodiversity (*Giupponi et al.*, 2006; *Marini et al.*, 2009). At the regional scale, the substitution of small farms with large and intensive farms is causing eutrophication of flat areas and abandonment of steep extensively-managed areas, with negative consequences on plant and insect diversity. Traditional animal husbandry should be maintained, as grassland management related to these systems demonstrated to support greater biodiversity than modern and intensive systems.

Local government are trying to address policy decision to sustain the multifunctional role of traditional extensive systems on the Alps. For example, since the '90 the Swiss government grants mountain farmers financial compensation for lower agricultural yields in exchange for landscape management and ecological services: ecological compensation area (ECA). The co-existence of well-managed pastures and long-term ecological compensation area (meadows) might best conserve mountain grassland biodiversity (*Kampmann et al.*, 2008).

**Table 4**

**Effects of livestock abandonment on land use change and biodiversity**

Authors	Study area	Change in livestock systems	Effects on land use and biodiversity
<i>McDonald et al.</i> , 2000	Different study areas in Eastern and Western Alps, from France to Slovenia	Dairy farming in Eastern and Western Alps, Sheep in Eastern Alps (Tyrol and Triglav): Intensification in conjunction with abandoning	Negative
<i>Giupponi et al.</i> , 2006	Belluno, Eastern Italian Alps	Decreasing of farms/km <sup>2</sup> UAA and increasing of cattle/farm. Less farms concentrated in the most favourable valleys with larger herd size.	Negative
<i>Gellrich et al.</i> , 2007	Swiss Alps	cultivation, cattle, sheep and goats. 1965-1990 farms number decreased (33%), average farm size increased.	Negative
<i>Kampmann et al.</i> , 2008	Eastern Central & Northern Alps of Switzerland	Grants for ecological services	Positive
<i>Marini et al.</i> , 2009	Trento, Eastern Italian Alps	1990-2000: number of farms decreased by 50%, the mean number of LU per farm increased from 16 to 26.	Negative

In general, agricultural and livestock activities in mountain areas must be evaluated not only for their economic viability, but also for: limitation of soil erosion, diversification of sources of income in agricultural farms, increasing of motivation and satisfaction for the farmer. Animal husbandry is also important for the tourism, because for many people the mountain natural environment is strictly related to livestock presence. Traditional practices survive in some areas only because of the requirements associated with the production of specialist cheeses (and the associated higher price of the milk), but in many areas EU hygiene and health standards for producing and marketing milk and dairy products, and the structural requirements for dairies, are causing problems for both producers and the competent authorities. There is a risk that these enterprises will be regarded as illegal leading to further abandonment of mountain pastures with associated loss of biodiversity and the increase in shrub/forest.

Also local policies must be addressed to pay for the environmental services of traditional livestock systems, which if fundamental for the maintenance of ecosystems and mountain culture, both seriously endangered during the last decades. Regional policies based on subsidies risks to be no longer sustainable, and future new strategies aimed to pay for product quality and environmental services of mountain farms must be developed to support the extensive livestock systems on the Alps.

## CONCLUSIONS

The evolution of livestock systems on the Alps shows that the abandoning of traditional practices cause a loss of natural resources in mountain areas. Traditional extensive systems did not guarantee economic viability; on the other hand, intensification is a losing strategy in mountain area, because the higher costs for feeding and services with respect the lowland and the impossibility to reach the same productive results. The sustainability of traditional, extensive farming should be promoted only through a “multifunctional farming” approach. The lower productivity of extensive production practices should be compensated by increasing the farm revenues through direct processing and marketing of products (on farm or in cooperation with other farmers), such as DOP cheeses. In the most favourable areas, also agro-tourism activities should be an opportunity to diversify the incomes; but in this case the risk is to lose the original agricultural vocation, with negative effect on grassland maintenance.

Finally, local policies must sustain traditional extensive systems paying for the landscape conservation and use of environmentally friendly practices. System dynamic studies become essential, not only to understand the changes that occurred in the past and identify what were the main drivers but also to forecast the changes that might happen in the future. If we can anticipate the evolution of farming systems, we might as well be able to support the design of more effective policies.

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## Organic livestock production in Slovenia

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### **ABSTRACT**

*Organic livestock production in Slovenia has oriented on milk and meat production with ruminants. Almost 90% of all agricultural area included in organic production belongs to livestock production on grassland. Slovenia has officially entered in the organic production close before year 2000. The trend of organic production from 2004 til 2009 is increasing, the most enlargement is on Equidae, Sheep, Pigs and Goat animals: 236, 199, 174 and 161% respectively. The organic surface present in Slovenia 6% of total used agricultural area.*

(Keywords: agriculture, livestock, organic farming)

### **INTRODUCTION**

Slovenia with 468,496 hectares of utilised agricultural area contributes 0.20% to the total surface area of the agricultural land of the EU-27 members. Natural conditions in Slovenia are relatively disadvantageous for agriculture. Approximately 60% of the total surface is covered by forest. Over 70% of the farmland is classified as less favoured area; most of it is grassland and the proportion of arable land is relatively low. The macroeconomic importance of agriculture is relatively low, while the agricultural sector accounts for 6% of total employment. The share of the gross domestic product (GDP) from agriculture is less than 4% and is declining. However, the economic importance of agriculture is higher than these data indicate; it is an important developmental, social and political factor.

The share of agriculture in the gross domestic product (GDP) is slightly lower than the European average and totals just fewer than 2%. Despite the small share in GDP, agriculture's significance is greater than seems at first sight. The multipurpose roles of agriculture in the Slovenian rural area are no longer just words on paper; instead, it has become publicly recognised and with its specific results it is making progress. Similarly, agriculture's importance relative to other primary economic branches is not in significant and the same is true of its relationship with the secondary and tertiary sectors.

Meadows and pastures represent us much as 60% of agricultural land while more than 20% of fields are used for the production of feed (maize, root crops, green feed...). This indicates the great importance of animal breeding in Slovenia. Primarily, ruminants are being bred and, of these, the breeding of sheep and goats is very much on the increase although, on average, the number of livestock units (LU) per farm is falling (6.3 LU).

Animal breeding is also the most important agricultural branch in terms of value. The total value of agricultural output is € 959 million, in which animal breeding contributes € 550 million. Otherwise, Slovenia's contribution to the total value of agricultural output of the EU-27 countries is 0.20%, which is exactly the same as its share of its utilised agricultural area.

In Slovenia up to 68% agricultural area are defined as less favoured areas (LFA). These are hilly and mountainous areas, areas with karst features or other factors that

limit possibilities of farming. Only 10% of Slovenian municipalities have no LFA within their boundaries. In these areas, farms are entitled to subsidiaries which totalled € 40.6 million or 35.5% of the budget expenditures of the rural development policy in 2005.

## HISTORY

In the middle of last century agriculture was under the pressure of intensive production, mostly as a consequence of the post-war shortage and famine in Europe. The technologies that were developed were not always environmentally-friendly. In the major part of Slovenia farmers never introduced intensive technologies mostly due to the natural conditions. Therefore, Slovenian agriculture policy became oriented to supporting environmentally-friendly agriculture even before the country joined the EU. The trend towards extensive production that started at the end of the previous century has had an impact on the most of Slovenia's agriculture. The exceptions were low-land farms which in the same period oriented towards intensive production or breeding (MAFFI, 2010). Slovenia's agricultural structure reflects the political and economic conditions of the socialist era. There were larger estates owned and run by the state and small private family farms. The latter owned over 90% of the total agricultural area. Nowadays, an average farm has only 5 hectares, and more than 85% of agricultural land is cultivated by farmers who hold less than 20 hectares.

Agricultural production has decreased in the last years. The most important branch is animal husbandry, especially dairy and beef production. Pig and poultry breeding are also important. Sheep breeding has increased quickly in the last few years. In the late 1980s and early 1990s, garden plot holders started an organic gardening movement, and in 1991, people interested in bio-dynamic farming formed a club. The latter developed into the bio-dynamic association AJDA. Very few farmers were involved in these initiatives; the majority of their members were garden plot holders and people not occupied in farming. Nevertheless, their activities were an important contribution to the development of organic farming in Slovenia.

In 1997, the Slovenian Organic Farmers' Association (S.O.F.A.) was founded. This was the first national association of pioneer organic farmers who were producing for the market, and who were therefore interested in the development of a certification system. The association adopted the standards for organic agriculture in Slovenia that were prepared by the Institute for Sustainable Development (ISD), a non-governmental organisation (NGO), specifically for this purpose. These standards were published by the Slovenian Ministry of Agriculture. They were prepared in accordance with the IFOAM Basic Standards and are similar to the standards of Austrian and German organic farmers' associations (Ernté and Bioland). In year 1998 join 44 farms, in year after 315 farms and in year 2009 is included 2096 farms (*Table 1*). In 1999, a total of 300 farms applied for certification under the new joint programme, and 22 applied for certification under the Demeter programme. Again, some farms were certified under both programmes (*Table 1*).

The area under organic agriculture increased significantly in the last years. In the period 2000–2009 area under organic production increase in the EU27 from 4.25 up to 8.50 mio ha or 7.5% per year. In Slovenia has increasing organic production more than 20% per year. In *Table 2* we can see that in last year most of the organic area represented permanent grassland with more 86%. Arable land represented only 10%, whereas orchard, vineyards and olive groves together less than 4%.

**Table 1**

**Increase in the number of organic farms and organic area in Slovenia**

Year	Number of organic farms	Hectares (estimate)	Percentage of agricultural area
1998	44	400	0.05%
1999	315	3,000	0.38%
2000	600	5,500	1.10%
2005	1,718	26,830	5.20%
2009	2,096	29,500	6.26%

**Table 2**

**Structure of all and organic utilised agricultural area by land use categories in Slovenia in 2009**

Land use categories	All	Organic	Organic UAA=100%
Utilised agricultural area (UAA)	468,496	29,388	100.0
Arable land	175,189	2,922	9.9
Permanent grassland	267,304	25,433	86.5
Orchard plantations	8,783	780	2.7
Vineyards	16,086	204	0.7
Olive groves	910	50	0.2

Source: SURS, 2010

## **LEGISLATION**

In 1996, the Institute for Sustainable Development prepared a proposal for the first Slovenian standards for organic production and processing, in accordance with the IFOAM Basic Standards and the EU Regulation on organic farming (Council Regulation (EEC) No. 2092/91). It was intended to provide the already existing organic farmers with a framework for further development. This effort was supported by the state Farming Advisory Service. The standards were published by this service and the Ministry of Agriculture as "Guidelines for Organic Farming in Slovenia", but as a legally non-binding document. These standards have been adopted by all organic farmers' associations that have emerged since 1997.

Activities to regulate organic farming at the state level started in 1998. The "Slovenian Regulation on Organic Farming" is expected to be adopted by the end of 2000 at the latest. The Ministry of Agriculture made this commitment in the context of EU pre-accession negotiations. The proposal for this regulation was prepared in accordance with Council Regulation (EEC) No. 2092/91. The basic act was established in 2002 and renewed in 2008 (The Agriculture Act (Uradni list RS, št. 54/2000 and Uradni list RS, št. 45/2008), the Council Regulation (EC) 834/2007 on organic production and labelling of organic products and import of organic products was implemented in year 2007, and Council Regulation (EC) 889/2008 was implemented in September 2008. In December 2008 Ministry of Agriculture, Forestry and Food adopted the Action plan of organic development in Slovenia to 2015. In year 2001 established the Rules of organic

farm production of food in agricultural (Uradni list RS, .t. 31/2001) and rules of condition for controlling organisations in organic production of food in agriculture.

## SUBSIDIES

Though the organic farming in Slovenia has started in late 80, the producers have gotten first subsidies in 1999 in form of direct payment per hectare for different crops. In year 2001 Slovenia has accepted Slovenian agricultural-environmental programme, which enable the organic farmers to get direct payments for measurements from this programme. This has further developed so that now the organic farmers can get direct payments for different productions (*Uredba o plačilih ...*, 2010). In *Table 3* direct payments for different productions are presented. They varied from 213.2 €/ha grassland per year to 578.92 €/ha vineyards hop field and nurseries per year. Direct payments are referring to an assurance of higher nutritional and environmental standards in food production.

**Table 3**

### Direct payment for different organic production in Slovenia productions

Production	€/ha per year
Arable land	298.07
Horticulture outside	551.45
Horticulture in covered area	487.90
Orchard plantations with at least 100 trees for nuts and chestnuts and 200 for other orchard and 150 olive groves per ha	554.73
Extensive orchards with 50-200 trees per ha	237.8
Vineyards, hop fields, nurseries	578.92
Grassland	
0.2–0.5 LU/ha	213.20
0.5–1.9 LU/ha	227.55

Source: *Uredba o plačilih ...*, 2010

## DEVELOPMENT AND ACTION PLAN UP TO 2015

Through the last decade the number of included animals in organic production has continuously increased. The number and development from 2004 to 2009 is presented in *Table 4*. The number of equidae in organic production has increased the most, for 236%, followed by sheep, pigs, poultry, goats and bovine animals. The number of bees has in the same time slightly decreased. The percentage of animals included in organic production from total number of animals varies significantly in different species. In pigs and poultry less than 1% of animals are included in organic production. In bovine animals it is around 4%, in dairy cows about 1%, whereas in suckler cows it is around 10%. Slightly higher is percentage of included equidae and the highest percentage is in sheep and goats with around one quarter of all animals included in organic production.

In *Table 5* the organic animal production in Slovenia from 2007 to 2008 is presented. Total organic meat production has tremendously decreased due to decreased beef production. On the other side pork, sheep, goat and poultry meat production has in the same time increased. Milk production did not change in the same time. Though the

increase was relatively high, especially in poultry meat, where the production of organic meat has increased for six fold, it is still very low. Organic beef production presented more than one percent, whereas pork and poultry much less than one percent of total production. Organic sheep and goat meat presented similar like the number of included animals in organic production about one quarter of total production. On average organic production represented lower percentage from total animal production than the number of included animals in organic production, which indicate that not all the animals are used for organic animal products.

**Table 4**

**Number of animals of different species included in organic production in Slovenia**

	<b>2004</b>	<b>2006</b>	<b>2008</b>	<b>2009</b>	<b>Index 2009/2004</b>
Bovine animals total	13,098	14,461	18,174	18,238	139
Dairy cows	1,004	920	1,086	1,172	117
Suckler cows	-	4,659	5,554	6,618	142*
Pigs	1,235	1,740	2,543	2,149	174
Sheep	17,946	22,920	36,107	35,751	199
Goats	3,465	3,964	6,339	5,569	161
Poultry	14,218	15,107	20,090	21,904	154
Equidae	1,178	1,856	2,486	2,784	236
Bees number of hives	2,072	2,098	1,592	1,912	92

Source: *Eurostat*, 2010

\*Index 2009/2006

**Table 5**

**Organic animal production in Slovenia**

	<b>2007</b>	<b>2008</b>
Meat total, t	1161	1040
- beef, t	809	676
- pork, t	30	43
- sheep and goat, t	315	294
- poultry, t	4	28
Milk, t	2678	2646

Source: *Eurostat*, 2010

## **CONCLUSIONS**

In Slovenia at the moment the demand on organic products is still greater than supply. Fruits and vegetables are in the greatest demand, though most of the Slovenian organic farmers are oriented in organic animal production, because of natural conditions. A lot of farmers decided for organic production because their former way of production was similar or near organic agricultural production. Most of the farmers market their products directly at home or local markets in bigger cities. On this way from animal

products only eggs, honey and some dairy products are marketed. For other products of animal origin and for farmers with larger production, which is not suitable for marketing on local markets, other market ways have to be further developed.

Organic animal production is not important only from healthy food production; preservation of natural resources, biodiversity and animal welfare point of view, but it helps and contributes also to sustainable development of rural areas. This might be in the future even more important as today and the role of organic agriculture production will further increase.

The most part of Slovenian livestock farming, especially in cattle, sheep and goat production is close to organic production, but not really officially declared for different reason. Lot of organic product is not on the market, because self consumption on farm, especially in small units like hobby farmers, which has more than 50% of agriculture land. The possibilities to marked the organic product is bigger than production, so that Slovenia is oriented to import the products from other countries.

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## **SECTION 1**

### **PIG BREEDING**





## Effect of weight, sex and age on technological quality of meat in Krškopolje pigs

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### ABSTRACT

*The effects of weight, sex and age on the technological quality were investigated. Forty-two Krškopolje pigs were included in experiment, 19 barrows and 23 gilts. Pigs were divided in two groups. One group had the average live weight 125 kg (G125) and the other 155 kg (G155). Age at slaughter varied from 236 to 364 days. Technological quality traits were measured in *M. longissimus dorsi* (LD) and *M. semimembranosus* (SM). pH value and conductivity were determined 45 min and 24 hrs after slaughter in LD and SM. Drip loss was observed after 24 and 48 hrs. Instrumental and visual colours were measured 24 hrs after slaughter in LD. Intramuscular fat content was analysed in LD. Statistical analysis was carried out by GLM procedure in SAS/STAT. G125 had lower pH measured 45 min after slaughter in LD compared to G155. Sex did not influence the technological quality traits. Older pigs had lower pH measured 24 hrs after slaughter in SM. Conductivity in LD measured 45 min and 24 hrs after slaughter and drip loss after 48 hrs were higher in older than in younger pigs. Furthermore, older pigs had more reddish colour and better visual evaluation of LD. In conclusion, younger Krškopolje pigs had better technological quality in comparison to older pigs with exception of colour.*

(Keywords: Krškopolje pig meat, technological quality, weight, sex, age)

### INTRODUCTION

Technological quality is very important for meat processing. It is determined by technological traits such as pH value, electrical conductivity, drip loss and colour. Firstly, the effect of pH value and electrical conductivity on technological quality was confirmed by *Blendl et al.* (1991). Further, drip loss in pig meat industry is economically important stated *Otto et al.* (2004). Meat with high drip loss has unattractive appearance (*Ngapo et al.*, 2004). It has low consumer acceptance, which leads to poor sales results. In addition, meat colour, which can be effected by drip loss and pH value, often influences the consumer's choice of product (*Ngapo et al.*, 2004). Finally, intramuscular fat (IMF) content is in correlation with some technological quality traits (*Huff-Lonergan et al.*, 2002). Technological quality of indigenous pig meat is interesting research topic because of its special meat characteristics.

Krškopolje pig (KP) is Slovenian indigenous pig breed. It is known for good meat quality (*Šalehar*, 1994). KP is black, with white unbroken belt over the shoulders and down to both front legs. It has an average fertility, good growth ability and relatively high losses under the barren rearing conditions (*Šalehar*, 1994). KP was neglected from 1971 onwards (*Šalehar*, 1991). Due to small herd size, it was endangered by extinction. In 1990 breed reconstruction began by increasing the population size and setting up a breeding program. There was some uncontrolled integration of other breeds used for mating due to

low number of sires (Šalehar, 1994). Latter on, breeding has focused on the elimination of undesired characteristics of other breeds. Production traits of KP were studied prior to neglect period by Ferjan (1969) and Eiselt (1971). Since the revival of this breed, Kastelic (2001) and Čandek-Potokar et al. (2003) investigated KP technological meat quality.

Indigenous pig breeds from other countries are also a common subject of research, where the effect of weight, sex and age on meat quality is analysed. Galián et al. (2009) found darker and less reddish meat 45 min after slaughter in lighter than in heavier indigenous Chato Murciano pigs. Lighter pigs had also darker and less yellowish meat 24 hrs after slaughter compared to the heavier ones. There were small differences between sexes in technological quality (Pugliese et al., 2004; Pugliese et al., 2005; Franci et al., 2005; Serrano et al., 2008). Moreover, the influence of age on meat quality of indigenous pig breeds is poorly researched.

The aim of the study was to determine the effect of live weight, sex and age at slaughter on technological quality traits of pig meat. Value pH, conductivity, drip loss, as well as instrumental and visual colour were observed. Intramuscular fat content was analyzed in the laboratory.

## MATERIALS AND METHODS

Forty-two Krškopolje pigs, 19 barrows and 23 gilts, were included in the experiment. They were housed in the same box with the same food composition and quantity for all pigs (Table 1). Feed composition was mainly wheat, barely and maize. Aftermath and water was available *ad libitum*.

**Table 1**

### Chemical feed composition

Feed composition	Feed (g/100 kg dry matter)	Aftermath (g/100 kg dry matter)
Crude protein	149.9	118.6
Crude fat	23.4	23.0
Crude fibre	47.8	286.1
Ash	39.7	100.1
Nitrogen-free extract	739.2	472.3
Phosphorous	4.5	3.6
Calcium	5.1	7.7
Magnesium	2.0	
Potassium	7.0	
Sodium	1.2	

Pigs were divided in two groups, due to comparison characteristics of heavier 155 kg (G155) and lighter 125 kg (G125) pigs. Slaughter was carried out in four groups. In each of them G125 and G155 were chosen. Slaughter age of G125 was between 236 and 364 days and G155 between 247 and 360 days.

After slaughter the warm carcasses were weighted. The pH was obtained by pH meter Metter Toledo (MA130 Ion Meter) in *M. longissimus dorsi* (LD) and *M. semimembranosus* (SM). It was measured 45 min (pH<sub>45</sub>) and 24 hrs (pH<sub>24</sub>) *post mortem*. The electrical conductivity was measured with conductometer LF/PT-STAR

(Matthäus) also in LD and SM 45 min and 24 hrs *post mortem*. Furthermore, drip loss was determined after 24 and 48 hrs by bag method (Honikel, 1998). Colour (L\*, a\*, b\*) was measured in LD cut at the last rib 24 hrs *post mortem* by Minolta Chromameter CR300 (Minolta Camera Co., Osaka, Japan). Visual colour was evaluated on the scale from 1 to 6 (Nakai, 1975). In addition, samples of LD were cut at the last rib and frozen to -20°C. Intramuscular fat content (IMF) was analyzed by the method of Folch *et al.* (1956).

Model used for statistical analysis was as follows (model 1):

$$y_{ijk} = \mu + G_i + S_j + b(x_{ijk} - \bar{x}) + e_{ijk} \quad (1)$$

where  $y_{ijk}$  is the observation value of the trait;  $\mu$  the overall mean;  $G_i$  the group class effect with two levels (G125, G155);  $S_j$  sex (barrows, gilts);  $b$  linear regression coefficient;  $x_{ij}$  age at slaughter included as covariate;  $\bar{x}$  the age at slaughter;  $e_{ij}$  the error. Statistical analysis was carried out using the GLM procedure with statistical package SAS/STAT (SAS Institute Inc., 2001). Differences between least square means were tested by the Tukey multiple test.

## RESULTS AND DISCUSSION

Technological quality traits of the KP did not show differences between weight groups and sexes (Table 2), except in pH<sub>45</sub>LD. G155 had higher value of the pH<sub>45</sub>LD (6.09) in comparison to G125 (5.91). Rapid decrease of pH value was the consequence of larger amount of lactic acid produced 45 min after slaughter. This process could lead to pale, soft and exudative (PSE) meat in G125. Heavier pigs usually had more marbling meat compared to the lighter pigs, which decelerate reduction of pH value. However, there was no difference in pH<sub>24</sub>LD (Table 2). The result was in agreement with Čandek-Potokar *et al.* (1997), where no differences in pH<sub>24</sub>LD between 100 and 130 kg live weight of hybrids Duroc×(Landrace×Large White) were found. They also determined no differences in pH<sub>45</sub>LD between lighter and heavier pigs. Furthermore, 100 kg pigs had more reddish (a\*), yellowish (b\*) and higher chromatic intensity (c\*) than pigs at 130 kg (Čandek-Potokar *et al.*, 1997), which is not confirmed with the results in this study (Table 2). Galián *et al.* (2009) reported darker (L\*) and less yellowish (b\*) colour of *M. longissimus lumborum* in lighter Chato Murciano pigs than in heavier ones. This is also not in agreement with this study, where the colour of G125 did not differ from G155 (Table 2). Furthermore, no difference in technological quality traits between sexes were found (Table 2) similarly as Fracni *et al.* (2005) and Pugliese *et al.* (2004) in Cinta Senese and Pugliese *et al.* (2005) in Nero Siciliano pigs.

Age at slaughter varied from 236 to 364 days and influenced the technological quality traits. The pH<sub>45</sub>SM and pH<sub>24</sub>LD were downward trend with age. Additionally, pH<sub>24</sub>SM was significantly lower in older pigs. Peinado *et al.* (2004) found higher pH<sub>45</sub>LD (6.39) and pH<sub>24</sub>LD (5.71) in 10 month old Chato Murciano pigs considering the results in Table 2. The KP had low pH value, which is not desired from nutritional point of view. However, it is still in lower limit of normal meat quality (Van Laack *et al.*, 1995). The consequence of higher formation of lactic acid is a lower pH value, which influenced the degradation of cell membranes. Such membranes did not hold the cell fluid. It led to worse water holding capacity and higher drip loss. Higher drip loss of LD after 48 hrs and trend after 24 hrs was determined in older than younger pigs (Table 2).

**Table 2****Least square means for group, sex and age effects on technological quality traits**

Traits	R <sup>2</sup>	Group		Sex		MSE	P-value			RC for age
		G125	G155	Barr.	Gilts		Group	Sex	Age	
pH45LD	0.16	5.91	6.09	6.00	6.00	0.05	0.0166	0.9809	0.7716	
pH45SM	0.14	6.04	6.10	6.07	6.08	0.06	0.4085	0.9019	0.0543	-0.003±0.002
pH24LD	0.10	5.45	5.47	5.46	5.46	0.01	0.5173	0.8307	0.0858	-0.001±0.000
pH24SM	0.49	5.42	5.42	5.41	4.42	0.02	0.9344	0.5786	<.0001	-0.002±0.000
Con45LD	0.25	4.52	4.23	4.52	4.23	0.17	0.3079	0.2620	0.0014	0.015±0.004
Con45SM	0.14	3.91	3.70	3.94	3.66	0.16	0.3835	0.2120	0.3872	
Con24LD	0.29	9.21	8.79	8.61	9.39	0.51	0.5962	0.2991	0.0035	0.039±0.013
Con24SM	0.21	7.34	6.66	7.56	6.43	0.56	0.4147	0.1482	0.1479	
Drip24(%)	0.11	3.05	2.47	2.79	2.73	0.56	0.1755	0.8593	0.0556	0.013±0.007
Drip48(%)	0.14	4.61	3.90	4.29	4.22	0.71	0.1690	0.8846	0.0219	0.019±0.008
L*	0.13	49.53	48.37	48.67	49.22	0.61	0.2590	0.5490	0.2691	
a*	0.13	10.77	10.58	10.65	10.71	0.26	0.6616	0.8731	0.0430	0.015±0.007
b*	0.11	5.76	5.47	5.44	5.79	0.21	0.3841	0.2482	0.1640	
c*	0.13	12.23	11.92	11.96	12.19	0.31	0.5504	0.6268	0.0519	0.017±0.008
h*	0.07	0.49	0.48	0.47	0.49	0.01	0.4409	0.1478	0.8560	
Colour	0.25	4.02	4.15	4.10	4.08	0.10	0.5824	0.9654	0.0178	0.007±0.003
IMF (%)	0.22	4.49	4.50	4.89	4.11	0.33	0.9869	0.1083	0.1218	

R<sup>2</sup>: coefficient of determination; MSE: mean standard error; RC: regression coefficient; G125, G155: groups with average body weight 125 kg, 155 kg; Barr: barrows, LD: *M. longissimus dorsi*; SM: *M. semimembranosus*; Con: conductivity; Drip: drip loss; Colour: visually determined colour on the scale from 1 to 6; IMF: intramuscular fat.

Results are in agreement with Čandek-Potokar et al. (1997) who found higher drip loss in 30 days older pigs than in younger ones. Muscles with higher drip loss, which means more mobile water, have usually higher electrical conductivity. So, conductivity measured 45 min and 24 hrs after slaughter was increased with age (Table 2). This signified worse meat quality of older pigs. However, it is still inside the limit for a normal quality 45 min after slaughter, where the limit is 8 mS/cm (Blendl et al., 1991). Electrical conductivity of meat in heavier pigs exceeded the limit for normal quality (9 mS/cm; Blendl et al., 1991) and represents danger for PSE meat. The colour of LD in older pigs was more reddish (a\*) and there was a trend in chromatic intensity (c\*) compared to younger pigs. Additionally, visual colour of LD had better evaluation in older than younger pigs. No influence of live weight, sex and age on the IMF content was observed.

## CONCLUSIONS

Weight and sex did not effect the technological quality of *M. longissimus dorsi* and *M. semimembranosus*. The exception was pH value measured 45 min after slaughter in *M. longissimus dorsi*, which was higher in heavier than in lighter pigs.

The study did not confirm the general opinion that older animals had better meat quality than younger ones. Older Krškopolje pigs had lower pH value, higher drip loss and limit value of conductivity. In conclusion, meat of older Krškopolje pigs was more liable to PSE quality.

The colour of LD was more reddish and better visually evaluated in older than in younger pigs.

The intramuscular fat content was not effected by weight group, sex nor age at slaughter.

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## Immunocastration of boars in Slovenian conditions

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### ABSTRACT

Four groups of pigs: boars (B), gilts (G), surgical castrates (SC) and immunocastrates (IC) were tested in groups with 24 animals between ages 80 and 167 days. The group IC was vaccinated against gonadotropin-releasing hormone with commercial Improvac® (Pfizer Inc.) vaccine at ages 70 and 130 days. The IC animals grew faster ( $1022 \pm 66$  g/day) than G ( $922 \pm 70$  g/day,  $P < 0.05$ ), SC ( $998 \pm 62$  g/day, N.S) and B animals ( $1044 \pm 87$  g/day, N.S). IC animals ( $54.83 \pm 4.35$  kg) produced more meat than SC animals ( $51.04 \pm 3.80$  kg,  $P < 0.05$ ). The feed:gain conversion ratio (kg feed per kg gain) was statistically significantly lower ( $P < 0.05$ ) in IC ( $2.99 \pm 0.18$ ) in comparison to the SC animals ( $3.23 \pm 0.28$ ). The income for every single pig was calculated according to Austrian paying scheme and based on 21-months average noted price. The feed costs and castration costs were calculated from the internal data of the company where the experiment was done and in the same time period. The total disadvantage of SC comparing to G animals was 11.70 EUR. The immunocastration would cover that gap. The difference between the G and IC animals is only 0.35 EUR in advantage of G animals. The results show enormous advantage of immunocastration, but inadequate realisation of relatively complicated subcutaneous application can cost all the advantage of immunocastration, because of boar taint.

(Keyword: pig, immunocastration, profitability)

### INTRODUCTION

Individually penned boars grow faster and are leaner than barrows due to anabolic effect of testicular hormones (Xue *et al.*, 1997). Pauly *et al.* (2008) found, that the boars eat less (1.88 versus 2.23 kg per day) and have better feed:gain conversion ratio (2.43 versus 2.69 kg feed per kg gain) than barrows. When the animals grow in a group, the sexual mature boars are socially more active, they eat less and grow more slowly than barrows (Cronin *et al.*, 2003). In some countries immature boars are slaughtered as normal fatteners, but not in Slovenia. The average slaughter weight of fatteners in Slovenia was 91.80 kg in year 2009 with an estimated meat percentage of 60.03 (Kovač *et al.*, 2010). Surgical castration of male pigs is a common praxis in Slovenian pig production.

The meat of sexually mature boars has specific taint. The immunization against gonadotropin – releasing hormone has a long term effect on hormonal profile (Zaramatskaia *et al.*, 2008). Immunised animals do not produce testosterone. Animals are called immunocastrates. The vaccination also eliminates the boar taint and improves growth performances of boars (Dunshea *et al.*, 2001). The consequence of immunocastration is faster and cheaper growth of leaner male animals without boar taint.

The positive effect on growth, feed:gain conversion and body composition of immunocastration was confirmed in many studies, but not in Slovenian conditions. The

aim of this study is to find out if the immunocastration of boars would improve the profitability of pig industry in Slovenia.

## MATERIALS AND METHODS

The experiment was done on Slovenian pig farm Ihan. It consisted of four groups: gilts (G), surgical castrates (SC), immunocastrates (IC) and entire males – boars (B).

Experimental animals were born in time interval of 14 days or two weeks. Animals were organised in two week groups. Fifty boars, twenty five castrates and twenty five gilts were selected every week. Animals were organised in two groups (first and second week of birth). Twenty four animals per group, twelve per week, were randomly chosen in the experiment before first vaccination. The IC group was vaccinated the first time with 2 ml IMPROVAC® (Pfizer Inc.) *subcutaneous* at the average age of 70 days. Animals were weighted first time at average age of 80 days. The second weighing was done at average age of 130 days and the third 37 days later at the age of 167 days or one day before slaughtering. At the second weighing (37 days before the end of the experiment or fifty days (seven weeks) after first vaccination) IC group was vaccinated the second time with the same quantity of vaccine. After first weighing animals were housed individually and fed *ad libitum*. Feed consumption was measured.

Two different feed formulations were used during the experiment: the first one between ages of 80 and 130 days (BEK 1) and the second between age of 130 days and the end of experiment (BEK 2). Both formulations based on maize and soya bean. BEK 1 consisted of minimum 16.5% crude proteins, minimum 1.00% lysine and 12.89 MJ of metabolic energy per kg feed. BEK 2 consisted of 15.0% crude proteins, 0.90% lysine and 12.60 MJ of metabolic energy per kg of feed. The feed prices in this study are from internal feed price list in the company.

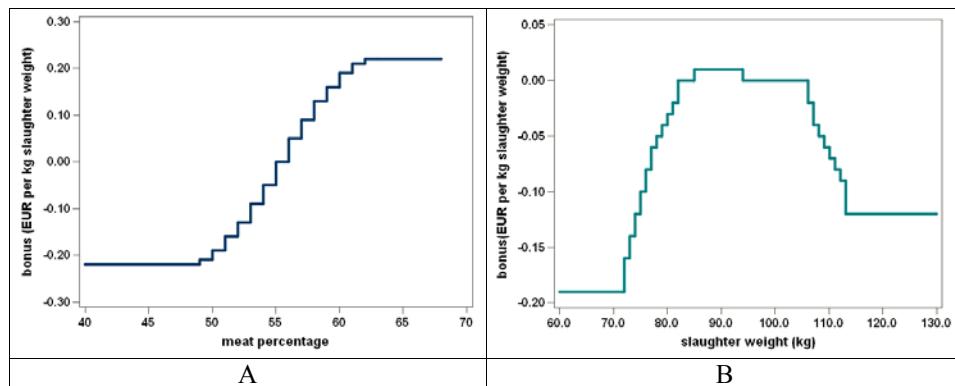
Animals were slaughtered at the end of the experiment. The carcasses were weighed just after slaughtering (slaughter weight). The meatiness of pigs was estimated according to Slovenian official method (*Pravilnik*, 2005). The revenue for every single pig was calculated according to Austrian noted price and Austrian paying scheme which are both widely used in Slovenia. Because of small production and because the majority of imported pig meat is coming from Austria and because a significant part of Slovenian pigs are slaughtered in Austria, pig prices are the same in Slovenia as in Austria. The bonuses for meat percentage and for slaughter weight according to Austrian paying scheme are presented in *Figure 1*.

*Figure 1* shows the bonuses on top of basic price. Basic price is calculated as noted price reduced for ten eurocents. The noted price is an estimated average price for kg of slaughter weight of pig, slaughtered in Austria. Austrian paying scheme prefers heavy pigs with slaughter weight between 82 and 106 kg. Lightweight pigs are heavily penalized. Pigs heavier than 106 kg are also penalized, but not so highly. The bonus for meat percentage increases between 56 and 63 percent of meat. The bonus for meatiness over 63% is the same as for 63% of meat. The price for pigs with meatiness under 56% is reduced. The noted price in this study is an average noted price between April 8<sup>th</sup>, 2008 and December 31<sup>st</sup>, 2009. The average price of feed was calculated in the same time period. Longer time interval would be better, but that period was chosen because the Austrian paying scheme was slightly changed on April 8<sup>th</sup>, 2008. The average noted price in that period was 1.431 € per kg slaughter weight. The average price BEK 1 (growth period between ages 80 and 130 days) was 0.199 and average price for BEK 2 (growth period between ages 130 and 167 days) was 0.180 EUR per kg feed. The feed

prices were relatively high in that period because of the high level of cereal prices on international market. The costs of surgical castration at the age of 3 days was estimated on 0.30 € per animal. The costs of immunocastration were estimated (IMPROVAC® and labour) on 3.70 EUR per animal.

**Figure 1**

**Austrian paying scheme (bonuses) for meatiness (A) and slaughter weight (B)**



The data were evaluated with SAS-STAT, procedure ANOVA program with simple 1-way model in which the effect of group (SC, IC, G and B) was included.

## RESULTS AND DISCUSSION

In the *Table 1* the results from analysis of variance and the group means tested with the Scheffe's multiple-comparison test are presented. Sales revenue for boars is hypothetical because boars are not classified as fatteners. The real price for boars is 0.60 EUR per kg slaughter weight. The calculated sales revenue for boars is used only for comparison of growth and feed efficiency of boars with the other groups of animals in the study.

The SC animals were at the age of 80 days slightly lighter than animals from other groups, but the difference was not statistically significant ( $P < 0.05$ ). At the end of experiment SC animals (117.1 kg) were heavier than G animals (112.1 kg) ( $P < 0.05$ ) and lighter than B (122.3 kg) and IC animals (121.0 kg,  $P < 0.05$ ). Slaughter weight shows the same pattern as weight of animals. The weight of the last two groups did not differ at that age. SC group grew slightly more slowly than B and IC groups, but the difference between those two groups was not statistically significant ( $P < 0.05$ ). Group G grew statistically significantly ( $P < 0.05$ ) more slowly than the other three groups (922 g per day comparing to B group with 1044 g per day, IC group 1022 g per day and SC group 998 per day). This can be explained with the small quantity of feed, which was consummated per day by G animals. Animals from group G (gilts) were in the last period of the experiment sexually mature. Faster growth of group B and better feed: gain conversion ratio comparing to G and IC group was often found (Dunshea *et al.*, 1993). It is expected, that the individually penned boars grow like in our experiment, where they grew faster than any other male castrates (Cronin *et al.*, 2003).

**Table 1****The probability for effect of group in model**

	Model group (P)	Mean (group)			
		B (Means for groups boars)	IC (Immunocastrates)	SC (Surgical castrates)	G (Gilts)
Weight 1, kg (at age 80 days)	0.4335	31.3±4.5a <sup>1</sup>	31.6±4.4a	29.8±4.1a	31.5±3.9a
Weight 2, kg (at age 167 days)	<0.0001	122.3±8.8a	121.0±6.7a	117.1±4.7ab	112.1±5.8b
Gain, kg (between ages 80 and 167 days)	<0.0001	90.9±7.3a	89.3±5.4a	87.3±5.7a	80.6±6.1b
DG, g/day (daily gain in the same period)	<0.0001	1044±87a	1022±66a	998±62a	922±70b
Slaughter weight, kg	0.0005	94.95±7.59a	93.31±6.01a	90.98±4.00ab	87.96±5.06b
Meat %	<0.0001	60.01±2.60a	58.75±2.36a	56.11±3.54b	60.33±2.76a
Meat, kg (quantity of meat)	<0.0001	56.93±4.52a	54.83±4.35ab	51.04±3.80c	53.05±3.60bc
DFC, kg (daily feed consumption in the period)	0.0007	2.94±0.31b	3.06±0.24ab	3.21±0.17a	2.98±0.19b
FCR (feed:gain conversion ratio in the period)	<0.0001	2.82±0.16b	2.99±0.18b	3.23±0.28a	3.24±0.22a
FC, EUR (feed costs in the period)	<0.0001	48.53±4.26b	50.68±2.96ab	53.18±3.40a	49.44±3.33b
FCC, EUR (feed and castration costs)	<0.0001	48.53±4.26b	54.38±2.96a	53.48±3.40a	49.44±3.33b
FC/kg, EUR (feed costs per kg gain in the period)	<0.0001	0.568±0.034b	0.568±0.031b	0.662±0.053a	0.614±0.042c
Revenue/kg, EUR (revenue per kg slaughter weight)	<0.0001	1.47±0.09a <sup>2</sup>	1.43±0.09a	1.33±0.13b	1.46±0.08a
Revenue / pig, EUR	<0.0001	138.99±11.56a <sup>2</sup>	133.38±12.80ab	121.14±13.39b	128.79±10.55ab

<sup>1</sup> the groups with the same letter are not statistically different (P<0.05); <sup>2</sup> the revenues for boars are hypothetical; the boars are not classified fatteners

Meat percentage of B (60.01), G (60.33) and IC animals (58.75) did not differ (P<0.05) between the groups. Meatiness was in that groups statistically significantly larger than in SC group (P<0.05). The effect of surgical castration was expected and positive. As a consequence of slightly higher slaughter weight (statistically nonsignificant) and higher meatiness, the quantity of meat was in IC animals more than 2.5 kg larger than in surgical castrates. The quantity of meat, produced by IC animals was smaller than the quantity, produced by B, but the difference was not statistically significant (P>0.05). The lower daily feed consumption of boars comparing to any other sex or castrates and better

feed:gain conversion ratio was found in most studies like *Fuchs et al.*, 2009, *Dunshea et al.*, 2001, and *Dunshea et al.*, 1993.

Differences between groups for sales revenue, feed costs (FC) and costs for feed and castration per pig (FCC) are presented in the *Table 2*.

The calculated hypothetical revenue of IC comparing to B animals was 5.61 EUR lower. The difference was not statistically significant ( $P < 0.05$ ). The revenue of IC animals was 4.95 EUR higher than the revenue of G and 12.94 EUR larger than the revenue of SC animals. The last difference was statistically significant ( $P < 0.05$ ). The revenue by gilts was also 7.64 EUR larger than by surgical castrates, but the difference was not statistically significant ( $P < 0.05$ ). If we suppose that half of the born animals are males and half females, the average price per kg slaughter weight would increase from 1.40 on 1.45 EUR and the average revenue per pig from 125.0 EUR for 6.1 EUR on 131.1 EUR. The turnover for the farm like Ihan, which produces 110 thousands pigs per year, would increase for more than 670 thousands EUR.

**Table 2**

**Differences between groups**

	Difference					
	IC-B <sup>1</sup>	IC-G	IC-SC	G-B <sup>1</sup>	G-SC	SC-B <sup>1</sup>
Sales revenue / pig	-5.61	4.59	12.24*	-10.20*	7.65	-17.85*
FC (costs for feed)	2.14	1.24	-2.51	0.90	-3.75*	4.65*
FCC (costs for feed and castration per pig)	5.84*	4.94*	0.89	0.90	-4.05*	4.95*
Total 1 (total benefit from revenue and feed costs (EUR))	-7.75	3.35	14.75	-11.10	11.40	-22.5
Total 2 (total benefit from revenue, feed costs and castration costs (EUR))	-11.45	-0.35	11.35	-11.10	11.70	-22.8

<sup>1</sup> the revenues for boars are hypothetical; \* the boars are not classified as fatteners

The effect of feed cost is much weaker. The feed costs for IC were 2.51 EUR lower than for SC group and 1.24 larger than in gilts. The differences were not statistically significant ( $P < 0.05$ ). When the costs for feed and castration were summarized (FCC), the differences between B and IC (B group 5.84 EUR less costs) and between G and IC (G group 4.94 EUR less costs) became statistically significant ( $P < 0.05$ ). The difference between B and IC groups is however only hypothetical because the boars in Slovenia are never categorised and paid as fatteners.

Differences between groups for summarized benefits from revenue and feed (Total 1) and for summarized benefits from revenue, feed and additional castration costs (Total 2) are presented in *Table 2* and *Figure 2*. The results are not statistically evaluated. The total benefit of immunocastration was compared to surgical castration +14.75 EUR (Total 1) or +11.35 EUR (Total 2). The benefit of immunocastration covered the gap between the SC and G groups; IC group and G group were on the same benefit level.

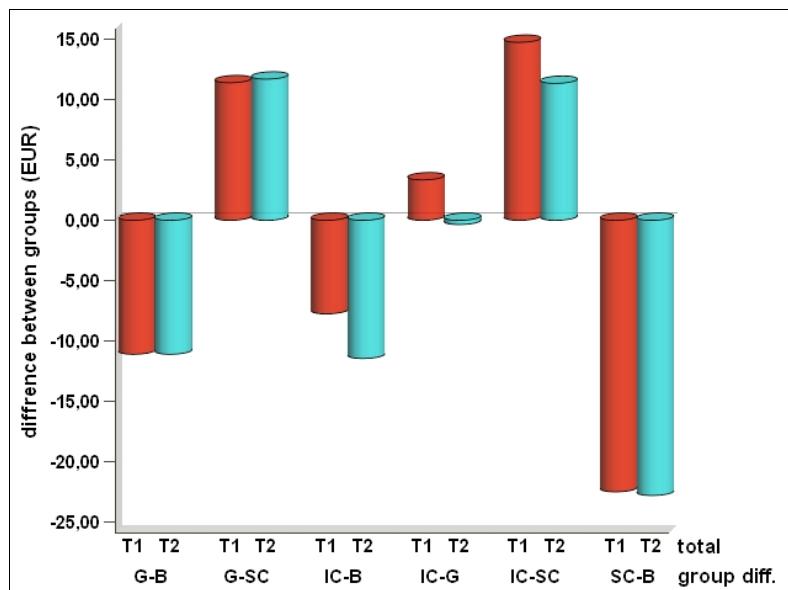
The results from the study show that the immunocastration is enormously superior method compared to classical surgical castration. The animals grow faster, they have better

(leaner) body composition and convert feed in body mass better than surgical castrates – more meat for less money. Unfortunately animals were not tested in production conditions where it is expected ten to twenty percent lower daily gain for castrates and gilts and adequate change of feed: gain conversion ratio. The experiment in production conditions without observation of individual feed intake will be done in the near future.

If the effect of castration will only be partial, the economic efficiency of immunocastration could be heavily affected. Such small batch of animals was very carefully injected. It is difficult to secure one hundred percent successful subcutaneous administration of vaccine in production conditions, where hundreds of animals would be treated daily. Only one smelling animal, classified as boar, would cost the benefit of seven to eight successfully immunocastrated animals. The reaction of consumers on immunocastration in Slovenia was not studied and is unknown.

**Figure 2**

**The differences between groups B, G, IC and SC for Total 1 and Total 2**



## CONCLUSIONS

Castration of male pigs is a common praxis in European countries where heavier pigs are slaughtered. The castration is a common praxis also in Slovenia. Surgical castrates grow more slowly than boars. They have higher feed:gain conversion ratio and are fattier than gilts. The new commercial vaccine against gonadotropin – realising hormone, Improvac® is already registered in European market. In an experiment where the gilts, boars, immunocastrates and surgical castrates grew in individual pens from age of 80 to 167 days, immunocastrates grew faster ( $P<0.05$ , 1022 g/day) than gilts (922 g/day). The daily gain of boars (1044 g/day) and surgical castrates (998 g/day) was not statistically different from the daily gain of the immunocastrates. Immunocastrates produced in live

period 2.5 kg more meat in carcasses ( $P<0.05$ ) than surgical castrates. The feed:gain ratio (kg feed/kg gain) in immunocastrates was only  $2.99\pm0.18$  comparing to  $3.23\pm0.28$  in surgical castrates. The means were statistically different ( $P<0.05$ ).

The income for every single pig was calculated according to Austrian noted price and Austrian paying scheme which are both widely used in Slovenia. The immunocastration has a positive effect on revenue and on costs per animal. When the costs of castration are included, the total benefit of immunocastration brings the efficiency of immunocastrates on the level of gilts. The total benefit of more efficient growth and better body composition of immunocastrates comparing to surgical castrates was 11.35 EUR per animal. Such benefit can be secured only if the realization of problematic *subcutaneous* administration is done successfully.

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## Genetic study of longevity of Hungarian pigs

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### ABSTRACT

*Authors jointly analyzed dataset measuring the reproductive performance of Hungarian Large White (LW, n=37806), Hungarian Landrace (LR, n=13620) and their cross (F1, n=40652). Number of records was 283528 collected for 92082 dams born between 1999–2004. Using the Weibull model (survival analysis) lifetime performances of the different genotypes were evaluated. The herd effects were significant for both lifetime performance traits (age at culling, total number of piglets born alive). The effect of age was only significant for total number of piglets born alive although the differences were small. Inbreeding on the other hand unfavourably affected lifetime performance for both traits.*

(Keywords: lifetime performance, inbreeding, swine, survival analysis)

### INTRODUCTION

Survival analysis is a well known and widely used statistical procedure correlating time to event data. An event might be death, developing a certain disease, or any other condition if its occurrence can be clearly detected on time scale. Survival analysis is mainly used in health sciences where the efficiency of various alternative treatments against some pre-defined illnesses can be compared with each other. Yet the method is widely used by sociologists, criminologists and also by animal scientists. The invaluable advantage of this procedure is that the so called censored data are not wasted but also used in the analysis. Censoring means that the event has not occurred till the experiment is terminated and thus the exact survival time of the participant is unknown. These phenomena either cannot be handled at all by other types of statistical analyses (like analysis of variance) or the data is reduced to being dichotomous (logistic regression) and hence considerable information is lost. Its application in the Hungarian pig breeding was practically absent until the beginning of the last decade (Nagy *et al.*, 2002) but more recently several studies were published (Balogh *et al.*, 2007a, 2007b; Balogh *et al.*, 2008). However, the effects of inbreeding were not yet been tested for any Hungarian pig population with the help of survival analysis. This was the objective of this study.

### MATERIALS AND METHODS

The analyses were conducted using the Hungarian Pig breeding dataset. Farrowing data containing 283528 records of 92082 dams born between 1999–2004 were evaluated. The dataset contained the reproductive performance of three genotypes: Hungarian Large White (LW, n=37806), Hungarian Landrace (LR, n=13620) and Hungarian Large White×Hungarian Landrace (F1, n=40652). The dams were originated from 193 herds and their lifetime parity number varied between 1 and 15. Lifetime performance was measured as either age (AGE) (until culling) or total number of pigs born alive (TNB)

pooling the litter sizes of the successive parturitions. Basic statistics of the lifetime performance of the analyzed genotypes is provided in *Table 1*.

**Table 1**

**Mean and standard deviation of age and total number of born piglets in the analyzed genotypes**

Genotype	LW		LR		F1	
Trait	AGE	TNB	AGE	TNB	AGE	TNB
Mean	784	31.9	738	31.4	760	30.8
Std.	385	24.5	359	22.7	370	23.2

Lifetime performance was evaluated the WEIBULL model (*Weibull*, 1951) using the SURVIVAL KIT software (*Ducrocq and Sölkner*, 1998). Four different models were applied. In the first two models the factors taken into the model were: genotype (3), herd (193) while the dependent variables were AGE and TNB, respectively. In the third and fourth models only the purebreds were analyzed but as an additional factor the inbreeding coefficient of the dams was also taken into the analysis. The calculations were done with the PEDIG software (*Boichard*, 2002) using the algorithm of Van Raden. The inbreeding coefficients (%) were classified into 7 levels (1: 0; 2: 0–3.125; 3: 3.125–6.25; 4: 6.25–12.5; 5: 12.5–18.25; 6: 18.25–25; 7: 25–)

**RESULTS AND DISCUSSION**

The results are presented in *Tables 2–5*. The results for the herd effects could not be presented in tables due to the high number of levels (193). Nevertheless significant differences were found across the herds for both traits. The effect of genotype on age was not significant while significant differences were found for the total number of born piglets, although the differences among the genotypes were small (*Tables 1–3*). Contrary to our results analyzing more than 10.000 records 6 different pig genotypes *Balogh et al.* (2006) observed significant differences among the genotypes' survival time (AGE) using the survival functions of *Kaplan and Meier* (1958).

After taking into account the inbreeding coefficients of the dams the genotype effects were not significant for either trait therefore were not presented (*Tables 4–5*). The herd effect remained highly significant while higher risk ratios could be observed (although only partly significant) for the inbred dams for both traits, thus inbreeding unfavourably affects lifetime performance (*Tables 4–5*). In a similar study made for several cattle breeds (*Sewalem et al.*, 2006) a trend toward increased risk of culling among more inbred animals was observed for all breeds.

**Table 2**

**Risk ratio of the dams depending on the genotype for AGE\***

Factor (Genotype)	Risk Ratio	Significance
1. Hungarian Large White	1.006	0.695
2. Hungarian Landrace	0.943	0.211
3. Hungarian Large White×Hungarian Landrace	1.000	*

\*AGE: age at culling

**Table 3**

**Risk ratio of the dams depending on the genotype for TNB\***

Factor (Genotype)	Risk Ratio	Significance
1. Hungarian Large White	1.034	0.027
2. Hungarian Landrace	1.021	0.665
3. Hungarian Large White×Hungarian Landrace	1.000	*

\*TNB: Total number of piglets born alive (pooled)

**Table 4**

**Risk ratio of the dams depending on the inbreeding coefficient for AGE\***

Factor (Inbreeding coefficient)	Risk Ratio	Significance
1. 0	1.000	*
2. 0–3.125	1.262	0.0000
3. 3.125–6.25	1.237	0.0000
4. 6.25–12.5	1.159	0.0141
5. 12.5–18.25	1.186	0.4128
6. 18.25–25	1.583	0.2008
7. 25–	1.146	0.0677

\*Age at culling

**Table 5**

**Risk ratio of the dams depending on the inbreeding coefficient for TNB\***

Factor (Inbreeding coefficient)	Risk Ratio	Significance
1. 0	1.000	0.0000
2. 0–3.125	1.168	0.0000
3. 3.125–6.25	1.198	0.0095
4. 6.25–12.5	1.169	0.6807
5. 12.5–18.25	1.090	0.4447
6. 18.25–25	1.309	0.0862
7. 25–	1.137	0.0000

\*TNB: Total number of piglets born alive (pooled)

## CONCLUSIONS

Based on the results it can be concluded that survival analysis can be successfully applied not only for evaluating lifetime performances but also for evaluating inbreeding effects for lifetime performing traits.

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## Analysis of fixed effects for the performance test traits of the Hungarian pigs

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### ABSTRACT

*Authors jointly analyzed station field and slaughterhouse datasets (collected and owned by the National Institute for Agricultural Quality Control between 2004.01.01 and 2009.04.30) of the Hungarian Large White, Hungarian Landrace pigs and their cross. Number of records were 5 396, 193 813) and 13 165 for station, field and slaughterhouse tests, respectively. Using multi-trait animal models the magnitude of the most important fixed effects (sex, genotype, herd, station, slaughterhouse and operator) were estimated for proportion of the valuable cuts (station test), days of test (station test), age at the end of the field test and lean meat percentage (field-slaughterhouse test), respectively. Most fixed effects were substantial except for genotype and sex which were negligible and small respectively.*

(Kulcsszavak: fixed effects, BLUP, pig, performance tests)

### INTRODUCTION

The primary objective of the performance tests to provide reliable data which can be evaluated in order to obtain correct ranking among breeding animals (breeding animal candidates). Although the performance tests are planned to exclude the various effects as much as possible the appearance of these effects is inevitable. The objective of the present analysis was therefore to evaluate the magnitude of the environmental and other effects for examined traits (proportion of the valuable cuts, days of test, age and lean meat percentage) of the Hungarian Large White, Hungarian Landrace pigs and their cross in the course of their station, field and slaughterhouse tests.

### MATERIALS AND METHODS

The analysis was carried out using the joint dataset of the Hungarian Large White, Hungarian Landrace pigs and their cross in the course of their station ( $n=5$  396), field ( $n=193$  813) and slaughterhouse (13 165) tests conducted between 2004.01.01 and 2009.04.30.

#### Station test (progeny test)

For the purpose of the station test a castrate and a female from the same litter are sent to the station between the ages of 65–77 days (random selection is assured). Body weight (BW) of the animals at the age of 65 days should be at least 17 kg but not greater than 32 kg. After some preliminary adaptation period the test begins at the age of 80 days (body weight at this age is at least 23 kg) and ends with reaching the final weight of 105 kg.

Animals are fed *ad libitum* and penned individually. Days of test (DOT), proportion of valuable cuts (neck, shoulder, loin and ham) (VC%) were recorded. Moreover body weight is measured at the beginning and at the end of the test with an accuracy of 1 kg.

#### Field test (own performance test)

In the field test ultrasonic (SONOMARK 100) fat depth measurements were taken from boars and gilts between 80 and 110 kg between the 3<sup>rd</sup> and 4<sup>th</sup> lumbar vertebrae (8 cm laterally from the spinal cord), between the 3<sup>rd</sup> and 4<sup>th</sup> ribs (6cm laterally from the spinal cord) and the loin muscle area between the 3<sup>rd</sup> and 4<sup>th</sup> ribs (6 cm laterally from the spinal cord). Using these measurements lean meat percentage (LMP) can be calculated. Age (AGE) and body weight (with an accuracy of 1 kg) of the animals were recorded at the same time. Gilts are kept in groups up to 25 pigs while boars are raised in smaller groups up to 15 on an *ad libitum* feeding regime.

#### Slaughterhouse test (progeny test)

This performance test although was theoretically available for a long time became more frequent only during the last years due to the increasing costs of the station tests. In order to evaluate the sows and boars 2 and 50 progeny were sent to the abattoir, respectively. The hot carcass weight has to be in the range of 50–120 kg but in our trial the range was much smaller (cca 80–110 kg). The lean meat percentage (LMP) is estimated from the same composite traits described at the field test. During the analysis the lean mean percentage obtained from the field and from the slaughterhouse tests were treated as identical traits.

The evaluated traits were: age at the end of the test (station test, field-slaughterhouse test), proportion of the valuable cuts (station test), and lean meat percentage (field-slaughterhouse test). Descriptive statistics of the evaluated traits are presented in *Table 1*.

**Table 1**

**Descriptive statistics of the examined traits**

Trait	Minimum	Maximum	Mean	Std.
BW (kg) (Live weight at the end of the station test)	103	110	105.2	1.85
BW (kg) (Live weight at the end of the field test)	80	149	98.1	9.99
AGE (day) (Age at the end of the station test)	123	200	157.9	12.24
AGE (day) (Age at the end of the field test)	120	300	179.8	22.8
VC% (%) (Proportion of the valuable cuts (station test))	45,0	59,6	50.7	2.66
LMP (%) (lean meat percentage (field-slaughterhouse test))	50	65	57.7	1.87

The datasets were evaluated with VCE5 (*Kovac and Groeneveld*, 2003) and PEST (*Groeneveld*, 1990) softwares applying multi-trait animal model. The structure of the model (following *Farkas*, 2008 and *Nagy et al.*, 2008) is given in *Table 2*.

**Table 2**

**Structure of the multi-trait animal model**

Trait	Type	Level	DOT (days of test, station test)	VC% (proportion of the valuable cuts, station test)	AGE (age at the end of the field test)	LMP (lean meat percentage, field- slaughterhouse test)
BW (kg) (Live weight at the end of the station test)	C	1	x	-	-	-
Genotype (Hungarian Large White, Hungarian Landrace pigs and their cross)	F	3	x	x	x	x
Year-month (Year and month of the station test)	F	65	x	x	-	-
Sex (male, female, castrate)	F	3	x	x	x	x
Herd (Place of the field test)	F	79	x	x	x	x
Station (Place of the station test)	F	5	x	x	-	-
BW (kg) (Live weight at the end of the field test)	C	1	-	-	x	-
Year-month (year and month of the field test)	F	65	-	-	x	x
Operator (operator of the ultrasound device)	F	20	-	-	x	x
Abattoir	F	4	-	-	-	-/x
Litter (random litter effect)	R	83 305	x	x	x	x
Animal (additive genetic effect)	A	233 776	x	x	x	x

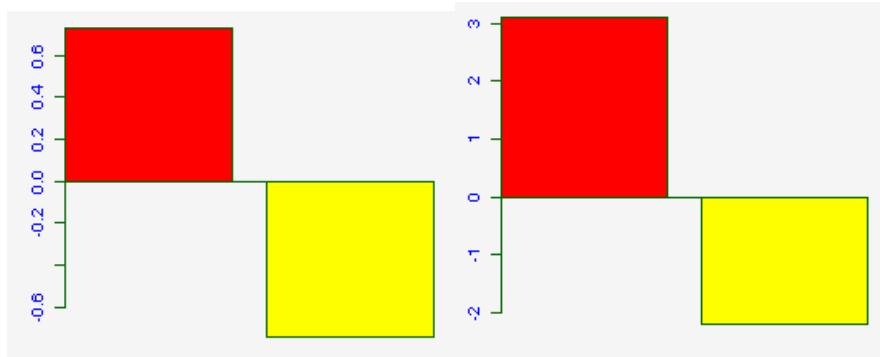
**RESULTS AND DISCUSSION**

Genetic parameter estimations were not the primarily objective of this study therefore the results are not provided. Magnitude of the sex effects are presented in *Figures 1–2*.

Male animals showed the most favourable performances for all traits. However the observed differences were relatively small. This result was in accordance with our previous study (Nagy et al., 2001). The genotype effects were very close to zero therefore these effects were not depicted.

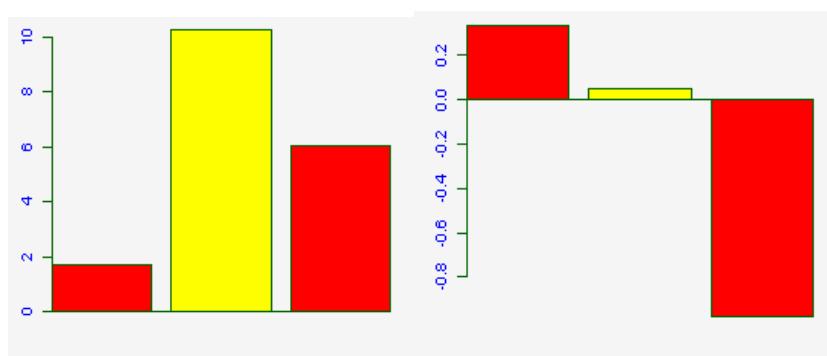
**Figure 1**

**Magnitude of the sex effects for proportion of the valuable cuts (station test) and days of test (station test), respectively (males vs. females; castrated vs. females)**



**Figure 2**

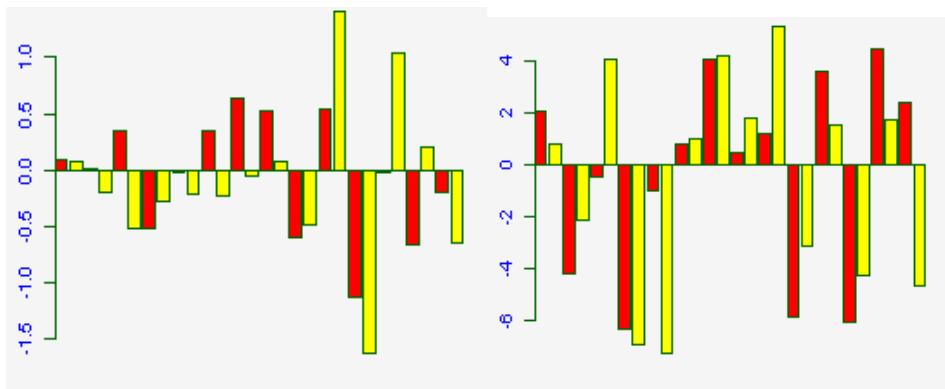
**Magnitude of the sex effects for age at the end of the field test and lean meat percentage (field-slaughterhouse test), respectively (males vs. females vs. castrated)**



The herd effects can be seen in *Figures 3–4*. Unlike the previous environmental factors the magnitudes of the herd effects were large for all traits but especially for the proportion of the valuable cuts and for age at the end of the field test. For these traits the magnitude of these effects reached the 25–40% of the phenotypic mean of the trait. This result also justified the relatively recently introduced BLUP procedure (MGSZH, 2009) and shows that with the conventional indices it could be impossible to evaluate the breeding candidate animals correctly but within herds.

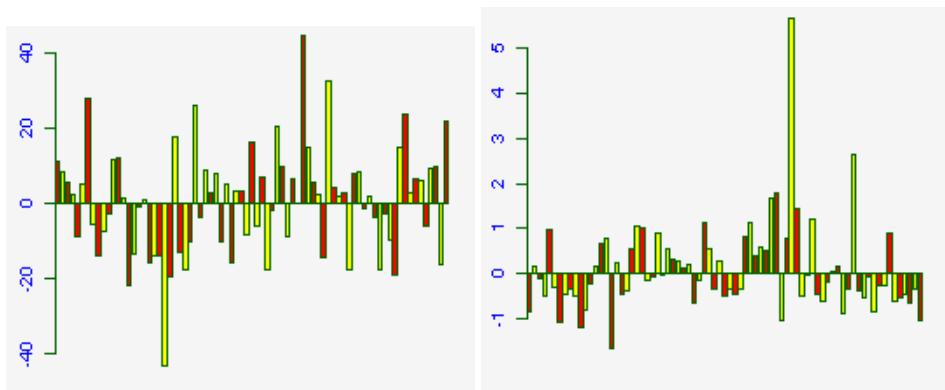
**Figure 3**

**Magnitude of the herd effects for proportion of the valuable cuts (station test) and days of test (station test), respectively**



**Figure 4**

**Magnitude of the herd effects for age at the end of the field test and lean meat percentage (field-slaughterhouse test), respectively**



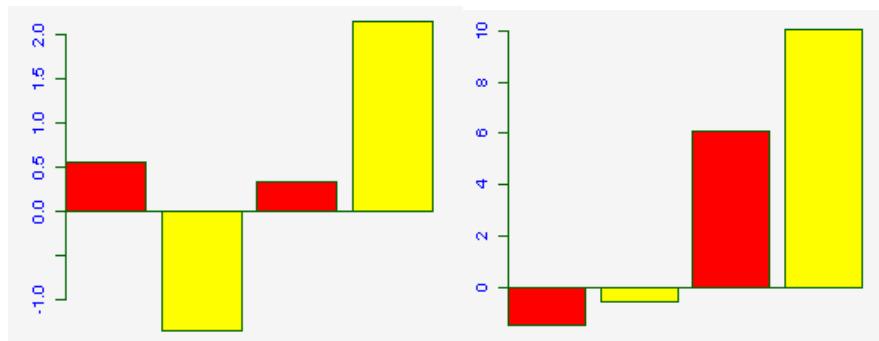
Station and slaughterhouse effects are shown in *Figures 5–6*. Due to the limited available space, the year-month effects are not depicted. For the valuable cuts and days of test the magnitude of the year-month effects were cca the same as the station. For age and for lean meat percentage the observed year-month effects were relatively small and they were about the same in magnitude as the sex effects. Contrary to Kovac and Groeneveld (1990) in the present study no regular cyclic pattern was not apparent for the year-month effects. Nagy *et al.* (2001) reported that the highest lean meat percentage values were observed during autumn meanwhile summer was the most disadvantageous season of all. However Nagy *et al.* (2001) also noted that the differences were negligible (1.63%). Tran *et al.* (1992) reported similar results.

The station effects were slightly higher in magnitude to that of the herd effects. The differences between these two effects were however small. Generally the station test is

planned to exclude the station effect as much as possible using the same penning and feeding regime (MGSZH, 2009). From the results it can be seen that this effort is partly reworded in the relatively small effects for the proportion of valuable cuts. Very similar station effects were found for percentage of valuable cuts by *Pescovicova et al.* (1999) as in this study. Nevertheless, substantial differences could be observed for days on test. This latter result also justifies the official introduction of the BLUP system and shows that even the most reliable station test would lead conventional index scores not fully capable for correct ranking of the animals across stations.

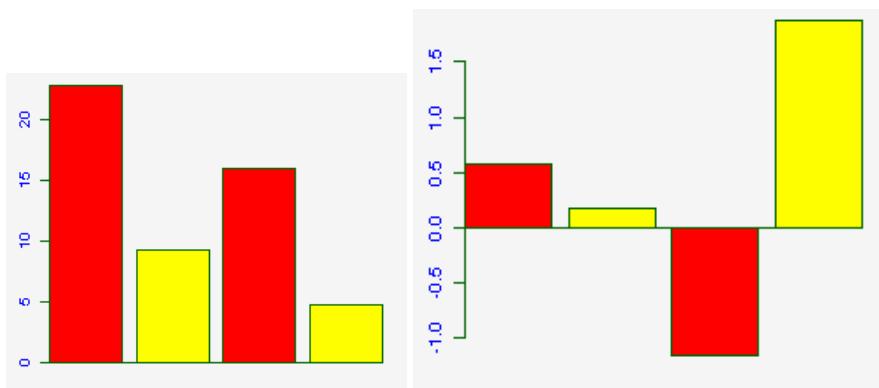
**Figure 5**

**Magnitude of the station effects for proportion of the valuable cuts (station test) and days of test (station test), respectively**



**Figure 6**

**Magnitude of the slaughterhouse effects for age at the end of the field test and lean meat percentage (field-slaughterhouse test), respectively**

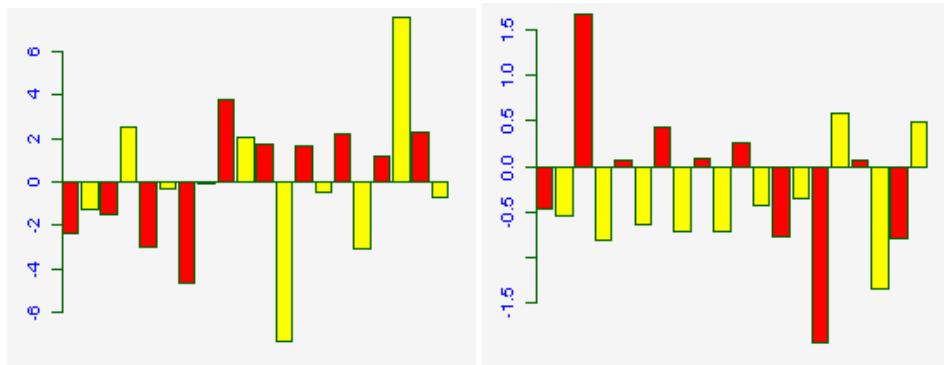


The slaughterhouse effects were similar in magnitude to that of the station tests. It has to be noted that although the ideal situation would be that the breeders could test their boars and sows at several abattoirs it is almost impossible due to market reasons. Non-partners

of certain can receive substantially lower price at another slaughterhouses thus evaluation of the slaughterhouses are probably suboptimal. The operator effects are provided in *Figure 7*.

**Figure 7**

**Magnitude of the operator effects for age at the end of the field test and lean meat percentage (field-slaughterhouse test), respectively**



Similarly to the abattoir effects the operators' evaluation would be optimal if several persons at the same time would evaluate the pigs within a herd and also if one operator would evaluate several herds. Unfortunately these criteria are not fully met. Nevertheless it is still very important to place the operators' code to the animal model because their personal care could probably be improved.

## CONCLUSIONS

Based on the results it can be concluded that genotype and sex effects were negligible and small while all other effects were substantial for the evaluated traits. Thus the conventional indices could not rank breeding animals properly and the recent official introduction of the BLUP procedure can be justified.

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## Estimation of genetic trend for the backfat depth of pigs of Large White breed in two Ukrainian pedigree farm

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### ABSTRACT

*The BLUP method is presently the most widely accepted method for predicting the genetic merit of pigs. However Ukraine is still among the countries where this selection method is awaiting for introduction. Therefore, our aim was to demonstrate the effectiveness of BLUP and REML methods on two selected pedigree farm data near Poltava. Analyses were carried out altogether on 1153 records (167 sires, 549 dams). Backfat measurements were recorded by Piglog 105 instrument over the 6/7 thoracic vertebra at the age of 6–7 months between 1997 and 2007. Due to the very small data size, the genetic parameters of the backfat depth were estimated for the Hungarian Large White dataset. Then with the help of these parameters, breeding values were estimated for the population of the present study using the PEST software applying single trait animal model. Least square means were highly stable for the examined period. Although the effect of year was significant ( $P<0.01$ ) the differences between the various years proved to be different only between 1994 and 1998 and between 1994 and 1999. Moreover the difference between the largest and smallest least square mean was less than 1 mm. Although the differences in the BLUE estimates were exceeding that of the GLM procedure (Figure 1) still it can be stated that the effects of the year-birth is relatively small, especially during the last 5 years. When the mean breeding values were regressed on the successive years the estimated annual genetic trend was 0.002 mm but it was not significant ( $P=0.15$ ). This result is unfavourable and suggests that the selection method used in the farms tested is inefficient in respect of backfat thickness.*

(Keywords: pig, BLUP, backfat)

### INTRODUCTION

The BLUP method (Henderson, 1975) is presently the most widely accepted method for predicting the genetic merit mainly due to its favourable mathematical properties (Kennedy *et al.*, 1988). The application of this procedure can be considered as a routine method and its first use in pig breeding was documented 25 years ago (Hudson and Kennedy, 1985). Since the most of the European countries officially introduced the method and the selection is based on BLUP breeding values (of various traits). The results of the BLUP selection were demonstrated by several authors (Kovac and Groeneveld, 1990; Chen *et al.*, 2002, 2003; Wolf *et al.*, 2005; Nagy *et al.*, 2008). However there are still some countries which still not implement BLUP selection and use the conventional Hazel indices. The reasons may be various but among these the most important might be the uninterested breeders and/or lack of the necessary

infrastructure (data collection systems etc). Ukraine is among the countries which still awaiting the official introduction of the BLUP procedure. Yet there is a clear intention towards that direction from the side of the Pig Breeding Institute, Poltava, Ukraine. The objective of the present study was to collect data from the nearby pig farms and analyze the collected data (for the first time) using BLUP and REML methods.

## MATERIALS AND METHODS

The genetic analysis was carried out using the data of 1153 female Large White pigs collected by the Pig Breeding Institute, Poltava between 1993–2007. The animals were originating from two herds (91, 1062), from 167 sires and 549 dams. During the measurements the backfat depth values were recorded using Piglog 105 device over 6/7 thoracic vertebra at the age of 6–7 months. The animals were kept in small groups up to 25 pigs on an *ad libitum* feeding regime. The basic statistics of the backfat depth is given in *Table 1*. Due to the unbalanced data structure across the subsequent years the least square means were calculated for the successive years using PROC GLM (SAS, 2004) taking into account the effects of year and month of birth and herds of origin.

**Table 1**

**Number of records, minimum, maximum values and standard deviation of the backfat depth (mm) measurements**

Year	N	Minimum	Maximum	Std <sup>1</sup>
1993	92	21	30	1.11
1994	97	21	30	1.18
1995	51	26	30	1.11
1996	21	27	29	0.75
1997	48	27	30	0.89
1998	129	26	30	0.75
1999	85	27	33	1.11
2000	63	27	30	0.82
2001	126	27	30	0.59
2002	50	27	29	0.47
2003	93	27	29	0.42
2004	35	27	28	0.41
2005	171	27	29	0.46
2006	70	27	28	0.12
2007	22	28	28	0.00
1993-2007	1153	21	33	0.82

<sup>1</sup> Standard deviation

Due to the very small data size, the genetic parameters of the backfat depth was estimated for the Hungarian Large White dataset described by *Csató et al.* (2002) using VCE5 (*Kovac and Groeneveld*, 2003). Then with the help of these parameters, breeding values were estimated for the population of the present study using the PEST (*Groeneveld*, 1990) software applying single trait animal model:

$$y = Xb + Za + e \quad (1)$$

Where:

$y$ = vector of observations,

$b$ = vector of fixed effects,

$a$ = vector of random animal effects,  $e$  vector of random residual effects,

$X$  and  $Z$  are incidence matrices relating records to fixed and random animal effects, respectively. Expected values of  $a$  and  $e$  were  $E(a)=E(e)=0$ . The variance-covariance structure assumed to be  $V(a)=\sigma_a^2$ ,  $V(e)=I\sigma_e^2$ , and  $\text{cov}(a,e)=\text{cov}(e,a)=0$ , where  $A$  is the numerator relationship matrix. Also  $\text{cov}(y,a)=ZA\sigma_a^2$ .

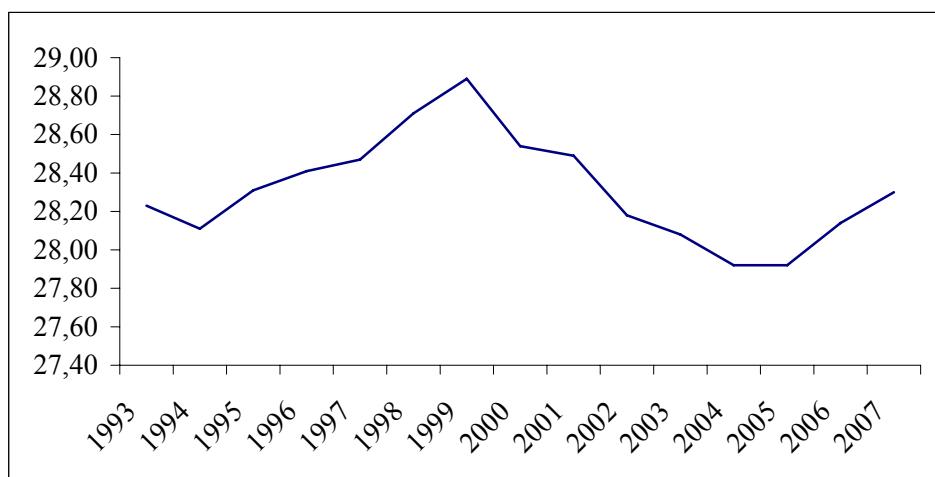
In the present study the year-month (of birth) and herd of origin were considered as fixed effects, while the additive genetic effects were treated as random effects.

## RESULTS AND DISCUSSION

The results of the GLM procedure is depicted in *Figure 1*.

**Figure 1**

**Least square means of the backfat measurements in the examined period**



It can be seen that the least square means were highly stable for the examined period. Although the effect of year was significant ( $P<0.01$ ) the differences between the various years proved to be different only between 1994 and 1998 and between 1994 and 1999. Moreover the difference between the largest and smallest least square mean was less than 1 mm. These values were similar to that of *Csató et al.* (2002) for backfat measurements recorded on the shoulder for Hungarian Large White (26.24 mm) and Hungarian Landrace (25.85 mm) pigs.

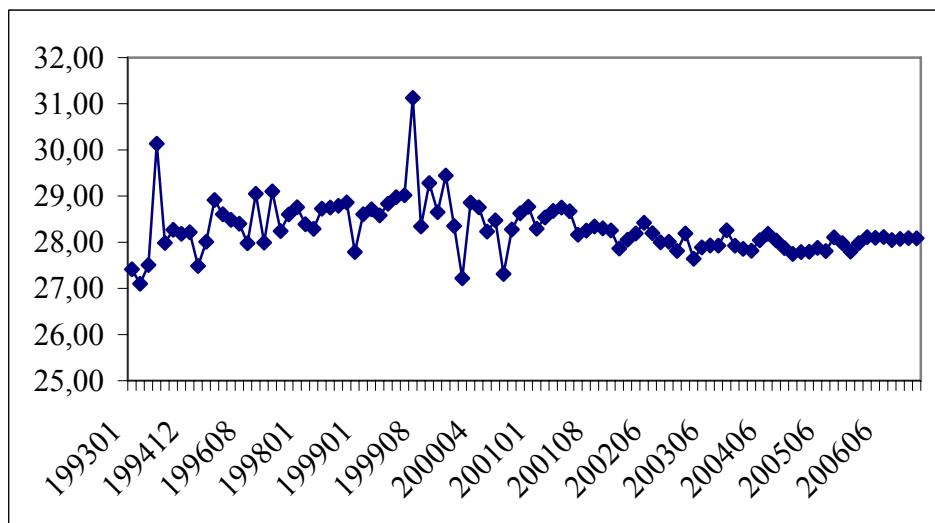
As reported by *Csató et al.* (2002) the heritability of the Hungarian Large White pigs was 0.35 for the backfat depth measured at the shoulder. Compared to the literature the value reported by *Csató et al.* (2002) was lower than that of *Clutter and Brascamp* (1998) reviewing 16 references. The average heritability for average backfat depth was 0.49 (the estimates ranged between 0.12–0.74) for these 16 studies which is moderately

high. It has to be noted that in Hungary other authors also found low heritability estimates for average backfat depth (Groeneveld *et al.*, 1996; Tran *et al.*, 1993). The reason for this difference between the Hungarian and international estimates might be that as suggested by Groeneveld *et al.* (1996) there might be some problems recording this trait. It has to be kept in mind also that generally the field test traits are much less precise than that of the station test traits. This was demonstrated by Sellier (1998) who found that the heritability of backfat was 30% higher when it was estimated in the course of a station test rather than in a field test.

The BLUE results for the Ukrainian population showed that there was only a small difference between the two groups of origin (1.19 mm) thus it can be presumed that the environmental differences were small between the groups. The BLUEs of the year-month effects are shown in the 2 *Figure*.

**Figure 2**

**BLUE estimates of the backfat measurements in the examined period**



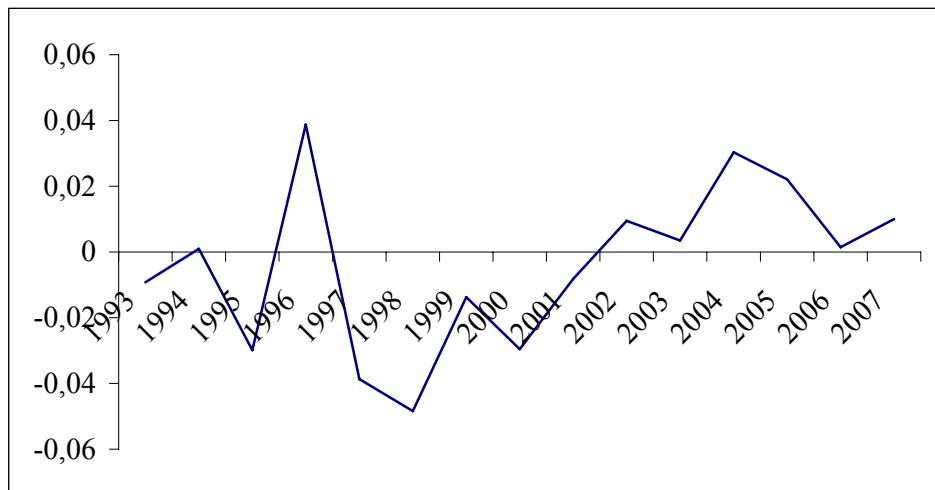
Although the differences in the BLUE estimates were exceeding that of the GLM procedure (*Figure 1*) still it can be stated that the effects of the year-birth is relatively small, especially during the last 5 years. The result suggests that probably no major changes in the technology were conducted at least during the last couple of years. The mean of the estimated breeding values (backfat depth) born in the same years are depicted in *Figure 3*.

When the mean breeding values were regressed on the successive years the estimated annual genetic trend was 0.002 mm but it was not significant ( $P=0.15$ ). This result is unfavourable and suggests that the selection method presently used in the farms tested for selection is inefficient in respect of backfat thickness improvement. On the contrary Hudson and Kennedy (1985) found an annual genetic trend of -0.12 mm for Yorkshire and -0.18 mm for Landrace. Groeneveld *et al.* (1998) and Wolf *et al.* (1998)

found similar values justifying that body composition can efficiently be improved by BLUP selection for backfat depth.

**Figure 3**

**Mean of the estimated breeding values (backfat depth) born in the same years**



## CONCLUSIONS

The results of the present study justify that although the data was not yet quite adequate for genetic parameter estimation using the parameters of a large dataset the breeding values still could be estimated. As expected no genetic trend was found because the selection was not based on the BLUP procedure. Further effort should be made to correct data across the herds near the Pig Breeding Institute of Poltava to introduce the BLUP procedure at least locally. Application of the BLUP procedure will have a positive effect on the selected traits. As a consequence other pig herds will hopefully also join those pig herds that are already selecting the breeding animals on BLUP breeding values thus the selection response will accelerate in the future in Ukraine.

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## **SECTION 2**

### **POULTRY AND RABBIT BREEDING**





## The effect of production type and age of hens on the major egg components

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### **ABSTRACT**

*The aim of the research was to find out the differences in the major egg components between egg and meat types of hens. Three traditional breeds of egg-type and three traditional breeds of meat-type hens were included into the experiment. The comparison was carried out at six different ages. Eggs from meat-type hens were significantly ( $P<0.05$ ) heavier, albumens, yolks and shells were heavier too while the proportion of yolk and diameter as well as yolk : albumen ratios were higher in comparison to egg-type hens. The proportion of albumen was significantly higher in eggs from egg-type hens while the thickness and proportion of shell did not differ between the types of hens. The last two mentioned traits were not significantly affected by age. The breed has a significant effect on all traits except percentage albumen and percentage yolk. In both types of hens the weights of albumen, yolk and shells were positively related to egg weight. The relation between yolk : albumen ratio and egg weight was positive but not significant in meat-type hens.*

(Keywords: egg-type hens, meat-type hens, egg components, yolk to albumen ratio)

### **INTRODUCTION**

An egg is a perfect creation of nature and its composition depends primarily on breed of hen, its age and nutrition. Despite the fact that eggs have been blamed due to cholesterol content the experts state that eggs contain a lot of nutrients (like vitamins of B complex, vitamin D, lecithin, unsaturated fatty acids, antioxidants) that diminish the negative effect of cholesterol. In a study with 40 thousand men and 80 thousand women *Hu et al.* (1999) proved that one consumed egg a day does not increase the risk of cardiovascular diseases and stroke in healthy women and men. Cholesterol and its esters are present in yolk (*Liu et al.*, 2010). The results of a few studies show that the amount of cholesterol is higher in eggs with heavier yolks and with a higher yolk to albumen ratio (*Hussein et al.*, 1993; *Campos*, 1995). Considering this fact the egg consumers should choose eggs with a lower yolk proportion and a higher albumen proportion. On the other hand, eggs with higher percentage yolk are appreciated in food industry that uses yolks as the basic component of some products, like mayonnaise (*Suk and Park*, 2001). The aim of the research was to find out the differences in proportions of main components (shell, albumen, yolk) in eggs produced by egg-type hens and meat-type hens.

### **MATERIALS AND METHODS**

Three Slovenian traditional egg-type breeds (Slovenian Brown hen, Slovenian Silver hen, Slovenian Barred hen) and three Slovenian traditional meat-type breeds (Slovenian

Early Feathered hen, Slovenian Late Feathered hen and Slovenian Fattening hen) were included into the experiment. All breeds were subjected to deep litter system rearing and fed on complete feeding mixture for egg-type laying hens. Egg-type hens were fed *ad libitum* while meat-type hens were fed restrictively. A random sample of 30 eggs was taken from each flock and measured on the same day. Samples of eggs were analyzed every four weeks, to begin with the 28<sup>th</sup> week. The last sample of eggs was taken at the age of 48 weeks. Each egg was weighted, broken and we separated the yolk from albumen. The yolk was weighted and its diameter measured. Egg shells with membranes were weighted and on three samples from equatorial part of egg we measured the thickness with a micro meter and calculated the average. Data on egg weight, yolk weight and shell weight served to calculate the albumen weight. We knew the weight of each egg as well as its components; therefore the weight of albumen, yolk and shell was expressed as the percentage of egg weight. Data were statistically processed by SAS/STAT software (SAS, 2003). For the regression and correlation analysis we used REG and CORR procedure, and GLM procedure for analysis of variance using the following statistical model:  $y_{ijk} = m + P_i + A_{ij} + B_{ik} + e_{ijkl}$  where:  $y$ = observed trait;  $m$ = mean population value;  $P_i$ = effect of production type  $i$ ;  $A_{ij}$ = effect of age  $j$  within production type  $j$ ,  $B_{ik}$ = effect of breed  $k$  within production type  $i$ ;  $e_{ijkl}$ = residue.

## RESULTS AND DISCUSSION

Table 1 shows the effects of production type of hen, its age and breed within the type on the studied traits. The production type and age showed a significant effect ( $P < 0.05$ ) on other traits except the shell thickness and shell proportion. *Basmacioglu and Ergul* (2005) did not report on significant effect of genotype on the shell thickness and shell proportion. Data on impact of hen age on the quality of egg shell often differ. *Silverside and Scott* (2001) and *Campo et al.* (2007) reported that the quality of shell worsen with aging while on the other hand *Yannakopoulos et al.* (1994) and *Van den Brand et al.* (2004) reported that hen age did not affect the shell thickness. The effect of breed within the production type was significant for most of the studied traits except the percentage albumen and percentage yolk where no significant differences were recorded between three meat-type and three egg-type hens (Table 1).

**Table 1**

**Statistical significance of three effects on some egg components (P-values)**

Trait	Production type of hen	Age within production type	Breed within production type
Egg weight (g)	0.0001	0.0001	0.0001
Albumen weight (g)	0.0016	0.0001	0.0001
Yolk weight (g)	0.0001	0.0001	0.0001
Shell weight (g)	0.0001	0.0001	0.0001
Shell thickness (mm)	0.3090	0.3894	0.0348
Percentage albumen (%)	0.0019	0.0001	0.4717
Percentage yolk (%)	0.0001	0.0001	0.1228
Percentage shell (%)	0.0510	0.2043	0.0001
Yolk : albumen ratio	0.0001	0.0001	0.0001
Diameter of yolk (cm)	0.0001	0.0001	0.0001

Differences between production types in each egg component, as well as the yolk : albumen ratio and yolk diameters are shown in *Table 2*. Eggs from egg-type hens have a low yolk : albumen ratio which is in comparison to the meat-type hens the result of relatively low yolk weight (*Table 2*).

**Table 2**

**The effect of production type of hen on egg components,  
on the yolk : albumen ratio and on the yolk diameter ((LSM $\pm$ SE)**

Trait	Egg-type	Meat-type
Egg weight (g)	58.28 <sup>a</sup> $\pm$ 0.18	61.89 <sup>b</sup> $\pm$ 0.20
Albumen weight (g)	36.45 <sup>a</sup> $\pm$ 0.16	37.20 <sup>b</sup> $\pm$ 0.17
Yolk weight (g)	15.19 <sup>a</sup> $\pm$ 0.05	17.76 <sup>b</sup> $\pm$ 0.05
Shell weight (g)	6.67 <sup>a</sup> $\pm$ 0.02	6.93 <sup>b</sup> $\pm$ 0.02
Shell thickness (mm)	0.376 <sup>a</sup> $\pm$ 0.014	0.354 <sup>a</sup> $\pm$ 0.015
Percentage albumen (%)	62.07 <sup>a</sup> $\pm$ 0.42	60.10 <sup>b</sup> $\pm$ 0.46
Percentage yolk (%)	26.41 <sup>a</sup> $\pm$ 0.31	28.66 <sup>b</sup> $\pm$ 0.34
Percentage shell (%)	11.58 <sup>a</sup> $\pm$ 0.11	11.24 <sup>a</sup> $\pm$ 0.12
Yolk: albumen ratio	0.414 <sup>a</sup> $\pm$ 0.002	0.480 <sup>b</sup> $\pm$ 0.002
Diameter of yolk (cm)	3.516 <sup>a</sup> $\pm$ 0.007	3.690 <sup>b</sup> $\pm$ 0.008

<sup>a, b</sup> Mean values within each line that do not have equal superscript are statistically significantly different at P<0.05

Eggs of meat-type hens significantly differed (P<0.05) from eggs from egg-type hens. Eggs had heavier albumens and yolks, yolk diameter was greater and shells were heavier (*Table 2*). Referring to previous studies (*Hussein et al.*, 1993; *Harms and Hussein*, 1993) the differences were expected. Eggs from meat-type hens contained 60.10% of albumen and 28.66% of yolk while eggs from egg-type hens contained 62.07% of albumen and 26.41% of yolk. Eggs from meat-type hens contained 1.97% less albumen and 2.25% more yolk than eggs from egg-type hens. In absolute amounts, the eggs from meat-type hens contained 0.75 g more albumen and 2.57 g more yolk than eggs from egg-type hens. Eggs from meat-type hens with heavier yolks and a higher yolk : albumen ratio contained more cholesterol (*Tavčar*, 2009).

Albumen weight as well as yolk weight and shell weight are positively related to egg weight even though the regression and correlation analyses show that the relation between albumen weight and egg weight is stronger ( $R^2$  0.80–0.85; coefficient of correlation 0.89–0.90) than the relation between egg weight and yolk weight ( $R^2$  0.49–0.56; coefficient of correlation 0.70–0.75) as well as the relation between egg weight and shell weight ( $R^2$  0.31–0.49; coefficient of correlation 0.55–0.70) (*Table 3*). *Harms and Hussein* (1993) and *Fletcher et al.* (1983) found out that the albumen weight is more related to egg weight than yolk weight. In egg-type hens we found a weak but significant relation between the yolk : albumen ratio and egg weight while this relation was not significant in meat-type hens. The relation between egg weight and yolk weight and proportion, respectively shows that a selection on higher egg weight within the egg-type hens results in higher yolk weight but a lower relative proportion in the whole egg weight. On the contrary, the selection to higher egg weight in meat-type hens increase the yolk weight and proportion in the whole egg weight (*Table 3*).

**Table 3****Regression and correlation analyses for some egg components with regard to egg weight**

Production type of hens	Trait	Regression analysis		Correlation analysis	
		Regression equation	P-value	Pearson coefficient of correlation	P-value
Egg-type	Albumen weight	$Y = -2.063 + 0.660x$ $R^2=0.85$	0.0001	0.90	0.0001
	Albumen proportion	$y = 23.514 + 0.659x$ $R^2=0.08$	0.0001	0.29	0.0001
	Yolk weight	$y = 1.079 + 0.242x$ $R^2=0.49$	0.0001	0.70	0.0001
	Yolk proportion	$Y = 53.828 - 0.470x$ $R^2=0.08$	0.0001	- 0.28	0.0001
	Shell weight	$y = 0.983 + 0.097x$ $R^2=0.49$	0.0001	0.70	0.0001
	Yolk : albumen ratio	$y = 0.213 + 0.003x$ $R^2=0.08$	0.0001	0.28	0.0001
Meat-type	Albumen weight	$Y = -0.621 + 0.610x$ $R^2=0.80$	0.0001	0.89	0.0001
	Albumen proportion	$y = 59.451 + 0.010x$ $R^2=0.0004$	0.6319	0.02	0.5720
	Yolk weight	$Y = -2.080 + 0.320x$ $R^2=0.56$	0.0001	0.75	0.0001
	Yolk proportion	$Y = 24.891 + 0.061x$ $R^2=0.01$	0.001	0.13	0.0018
	Shell weight	$y = 2.702 + 0.068x$ $R^2=0.31$	0.0001	0.55	0.0001
	Yolk : albumen ratio	$Y = 0.423 + 0.0009x$ $R^2=0.006$	0.0678	0.07	0.0714

The changes in studied traits that depend on age of a hen are shown in *Tables 4 and 5* for both production types of hens. In both types eggs were significantly heavier ( $P<0.05$ ) at the end of the trial (age 48 weeks). Yolks and shells were heavier, the yolk proportions were higher as well as the yolk : albumen ratio in comparison to eggs from the beginning of the trial (age 28 weeks) (*Table 4, 5*). Several authors reported on increase of the egg weight over the age of hens (*Johnston and Gous, 2007; Rizzi and Chiericato, 2005; Van den Brand et al., 2004*).

Contrary to above mentioned authors *Zemkova et al. (2007)* did not report that the hen age affected significantly the egg weight. The reason for a significant increase of the yolk : albumen ratio with age could be the fact that the yolk weight increased more quickly with hen age than albumen weight which did not significantly increase in the egg-type hens.

**Table 4**

**Weights of eggs, albumens, yolks and shells and shell thickness in two types of hens (LSM values)**

Age (weeks)	Egg weight (g)		Albumen weight (g)		Yolk weight (g)		Shell weight (g)		Shell thickness (mm)	
	Egg type	Meat type	Egg type	Meat type	Egg type	Meat type	Egg type	Meat type	Egg type	Meat type
28	57.38 <sup>a,A</sup>	56.49 <sup>a,A</sup>	37.02 <sup>a,A</sup>	35.21 <sup>a,A</sup>	13.97 <sup>a,A</sup>	14.70 <sup>b,A</sup>	6.53 <sup>a,A</sup>	6.57 <sup>a,A</sup>	0.362 <sup>a,A</sup>	0.362 <sup>a,A</sup>
32	58.09 <sup>a</sup>	58.83 <sup>a</sup>	36.70 <sup>a</sup>	36.03 <sup>a</sup>	14.72 <sup>a</sup>	16.19 <sup>b</sup>	6.66 <sup>a</sup>	6.60 <sup>a</sup>	0.356 <sup>a</sup>	0.344 <sup>a</sup>
36	59.69 <sup>a</sup>	61.04 <sup>a</sup>	37.45 <sup>a</sup>	36.81 <sup>a</sup>	15.64 <sup>a</sup>	17.34 <sup>b</sup>	6.77 <sup>a</sup>	6.89 <sup>a</sup>	0.352 <sup>a</sup>	0.348 <sup>a</sup>
40	60.45 <sup>a</sup>	63.35 <sup>b</sup>	37.58 <sup>a</sup>	37.74 <sup>a</sup>	16.11 <sup>a</sup>	18.45 <sup>b</sup>	6.75 <sup>a</sup>	7.15 <sup>b</sup>	0.497 <sup>a</sup>	0.357 <sup>a</sup>
44	60.92 <sup>a</sup>	65.24 <sup>b</sup>	36.91 <sup>a</sup>	38.61 <sup>a</sup>	16.77 <sup>a</sup>	19.68 <sup>b</sup>	7.23 <sup>a</sup>	7.02 <sup>a</sup>	0.362 <sup>a</sup>	0.361 <sup>a</sup>
48	61.91 <sup>a,B</sup>	66.37 <sup>b,B</sup>	37.26 <sup>a,A</sup>	38.81 <sup>a,B</sup>	17.58 <sup>a,B</sup>	20.18 <sup>b,B</sup>	7.06 <sup>a,B</sup>	7.37 <sup>b,B</sup>	0.353 <sup>a,A</sup>	0.351 <sup>a,A</sup>
SE	± 0.50	± 0.50	± 0.42	± 0.42	± 0.14	± 0.14	± 0.06	± 0.06	± 0.03	± 0.03

<sup>a-b</sup> Mean values within each line and trait that do not have equal superscript are statistically significantly different at P<0.05; <sup>A-B</sup> Mean values within each column (28<sup>th</sup> and 48<sup>th</sup> week of age) that do not have equal superscript are statistically significantly different at P<0.05

**Table 5**

**Albumen, yolk and shell proportions, yolk: albumen ratio and diameters of yolks in two types of hens (LSM values)**

Age (weeks)	Albumen proportion (%)		Yolk proportion (%)		Shell proportion (%)		Yolk : albumen ratio		Diameter of yolk (cm)	
	Egg type	Meat type	Egg type	Meat type	Egg type	Meat type	Egg type	Meat type	Egg type	Meat type
28	64.46 <sup>a,A</sup>	62.23 <sup>a,A</sup>	24.41 <sup>a,A</sup>	26.08 <sup>a,A</sup>	11.39 <sup>a,A</sup>	11.67 <sup>a,A</sup>	0.376 <sup>a,A</sup>	0.420 <sup>b,A</sup>	3.55 <sup>a,A</sup>	3.68 <sup>b,A</sup>
32	63.13 <sup>a</sup>	61.17 <sup>a</sup>	25.38 <sup>a</sup>	27.57 <sup>a</sup>	11.48 <sup>a</sup>	11.25 <sup>a</sup>	0.403 <sup>a</sup>	0.452 <sup>b</sup>	3.58 <sup>a</sup>	3.72 <sup>b</sup>
36	62.70 <sup>a</sup>	60.28 <sup>a</sup>	26.24 <sup>a</sup>	28.41 <sup>a</sup>	11.34 <sup>a</sup>	11.29 <sup>a</sup>	0.417 <sup>a</sup>	0.472 <sup>b</sup>	3.63 <sup>a</sup>	3.85 <sup>b</sup>
40	62.11 <sup>a</sup>	59.52 <sup>a</sup>	26.71 <sup>a</sup>	29.17 <sup>a</sup>	11.17 <sup>a</sup>	11.30 <sup>a</sup>	0.431 <sup>a</sup>	0.491 <sup>b</sup>	3.59 <sup>a</sup>	3.65 <sup>a</sup>
44	60.49 <sup>a</sup>	59.10 <sup>a</sup>	27.61 <sup>a</sup>	30.21 <sup>a</sup>	11.89 <sup>a</sup>	10.78 <sup>a</sup>	0.458 <sup>a</sup>	0.513 <sup>b</sup>	3.43 <sup>a</sup>	3.51 <sup>a</sup>
48	56.53 <sup>a,B</sup>	58.32 <sup>a,B</sup>	31.10 <sup>a,B</sup>	30.50 <sup>a,B</sup>	12.36 <sup>a,B</sup>	11.16 <sup>a,A</sup>	0.451 <sup>a,B</sup>	0.528 <sup>b,B</sup>	3.60 <sup>a,B</sup>	3.69 <sup>a,A</sup>
SE	± 1.13	± 1.13	± 0.83	± 0.83	± 0.30	± 0.30	± 0.007	± 0.007	± 0.02	± 0.02

<sup>a-b</sup> Mean values within each line and trait that do not have equal superscript are statistically significantly different at P<0.05; <sup>A-B</sup> Mean values within each column (28<sup>th</sup> and 48<sup>th</sup> week of age) that do not have equal superscript are statistically significantly different at P<0.05

Between the age 28 weeks and 48 weeks the albumen weight increased by 0.65% (egg type), 10.22% (meat type); while in the same period the yolk weight increased by 25.84% (egg type) and 37.27% (meat type). The absolute and relative amounts of yolk increased over hen age. The relative amount of albumen in egg decreased over the hen age. The result is in accordance with the studies of *Van den Brand et al. (2004)*, *Rizzi*

and Chiericato (2005) and Lukaš *et al.* (2009) who in different genotypes of hens confirmed the decrease of albumen proportion with increase of hen age. The percentage shell significantly increased over the age ( $P<0.05$ ) in egg-type hens while in the meat-type a lower decrease that was not significant was noticed. The mentioned finding is not in accordance with the report of Curtis *et al.* (1986) who in six lines of egg-type laying hens found a decrease of shell proportion over hen aging. In all ages the yolk : albumen ratio was significantly higher ( $P<0.05$ ) in eggs from meat-type hens. Their eggs were significantly heavier at the age of 40 to 48 weeks in comparison to eggs from egg-type hens.

## CONCLUSIONS

Due to higher ratio of yolk to albumen the eggs of meat-type hens ought to be used for such production where yolks are separated from albumens while eggs from egg-type hens ought to be used as table eggs in shells.

Within both types of hens the ratio of yolk to albumen increases over hen age, therefore eggs from younger hens of any type are more suitable for sale in shells while eggs of older hens could be used for processing.

The selection of hens to higher egg weight within the meat-type hens does not increase the ratio of yolk to albumen.

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## Population proteomics as a novel tool for breed characterization: practical considerations

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### **ABSTRACT**

*The aim of this study was to describe the pros and cons of a population proteomic approach aiming to characterize local chicken breeds. The experiment involved a total of 29 males of Pépoi, Padovana, and Ermellinata di Rovigo Italian local chicken breeds. Sarcoplasmic protein fractions of breast muscle were analysed by two-dimensional electrophoresis. Image analysis followed by statistical analysis enabled to differentiate groups of individuals on the basis of similarities of protein expression. Individuals well clustered into groups corresponding to the breed of origin. The Significance Analysis of Microarray analysis enabled identification of the most relevant spots regarding breed differentiation; 10 of these were identified by Mass Spectrometry, revealing preliminary evidences on the mechanisms of the breed differentiation process. The approach succeed in differentiating the individuals in groups corresponding to the different breeds, unfolding the relations among breeds and single individuals, analyzing and measuring the genetic variation at encoding loci. Results evidenced the ability to proteomic analyses to identify and to characterize chicken breeds.*

(Keywords: population proteomics, characterization, chicken, local breeds)

### **INTRODUCTION**

Proteomics is a well established technique for the separation and identification of proteins in complex samples. Although it has been used in the past for phylogenetic studies using bood or single protein polymorphisms (*Okabayashi et al.*, 1998; *Inafuku et al.*, 1998), only in recent years comparative proteomics techniques, in particular bi-dimensional electrophoresis, enabled the large-scale screening for hundreds proteins in a single step. It shows great potential in provinding highly valuable information in phylogenetic analysis, and has the ability to reveal new perspectives and lines of research (*Biron et al.*, 2006). Beyond that, proteomics complements and extends study of genomic and transcript data, reflecting true biochemical outcome of genetic information (*Doherty et al.*, 2007). Currently, just few authors used proteomics to investigate natural variation within species populations (*Biron et al.*, 2006), and despite the advances made in this discipline, there is a lack of algorithms and statistical tools for handling the impressive amount of information obtainable from such techniques (*Navas and Albar*, 2004). The aim of this study is to describe the pros and cons of a population proteomic approach aiming to characterize local chicken breeds.

### **MATERIALS AND METHODS**

In the Veneto region of Italy the local breeds of chicken, which are typically reared in free range systems, provide an interesting alternative to commercial lines. The trial made

use of day-old chicks of three Italian local chicken breeds: Padovana (PD), Pépoi (PP) and Ermellinata di Rovigo (ER). A total of 29 males (PD=10, PP=10, and ER=9) were slaughtered at 190 d of age. At hatch, chicks were placed together in an indoor pen with access to a grass paddock. Rearing, feeding, slaughtering conditions and veterinary treatments were the same for all animals. About 15 min post mortem, 5 grams samples of muscle (*Pectoralis superficialis*) were collected from the left breast and frozen in liquid Nitrogen for the analysis.

The extraction of sarcoplasmic proteins was performed using the procedure described by Rathgeber *et al.* (1999). This protein fraction represent about 30–35% of the muscular proteins. Despite the great diversity of this class of proteins, they share common characteristics such as a relatively low molecular weight, a relatively high isoelectric point and globular structure. A total of 58 samples were analysed by two-dimensional electrophoresis (2 repetitions per animal). Protein concentration was quantified using the Bradford assay (Bio-Rad). The isoelectric focusing (IEF) was carried out using a Protean IEF cell (Bio-Rad Laboratories Inc., Hercules, CA), loading 300 µg of protein each strip (17 cm, pH 4–7 linear). SDS-PAGE was performed in a Protean XL cell (Bio-Rad Laboratories) on 12% polyacrylamide gels (2.6% bisacrylamide) at 35 mA/gel at 8 °C, until the dye track reached the end of the gels. Gels were silver stained following the protocol of Shevchenko *et al.* (1996). Gels images were acquired through a GS-800 densitometer and analysed with a computerized image analysis: Image Master 2D Platinum (GE Healthcare). Spots were excised from gels, destained and digested at 37 °C for 5 h. Details about the used protocols are available upon request. A Voyager DE-Pro model MALDI-TOF mass spectrometer (PerSeptive BioSystems) was used in positive-ion reflector mode for PMF. External calibration was performed with a standard peptide solution (Proteomix). PMFs were compared to Aves nrNCBI (12/2008, 102 448 seq) protein sequence databases using MASCOT 2.2 software. The initial search parameters allowed a single trypsin missed cleavage, partial carbamidomethylation of cystein, partial oxidation of methionine and mass deviation lower than 25 ppm.

All spots detected were included for the statistical analysis. Cluster analysis was performed using the PROC CLUSTER of SAS (1997) and the Ward's minimum variance option. Dendograms were plotted using PROC TREE procedure of SAS. The statistical differences in protein expression among groups were tested using the Significance Analysis of Microarrays (SAM) method as described by Meunier *et al.* (2005). Spots with a Fold Change greater than 2 were retained and considered for the identification.

## RESULTS AND DISCUSSION

Image analysis detected 246, 275, and 226 different spots for the PP, PD and ER breeds, respectively, respectively, showing that PD has the highest proteomic richness. For each spot, expression results were averaged to obtain a single value within individual.

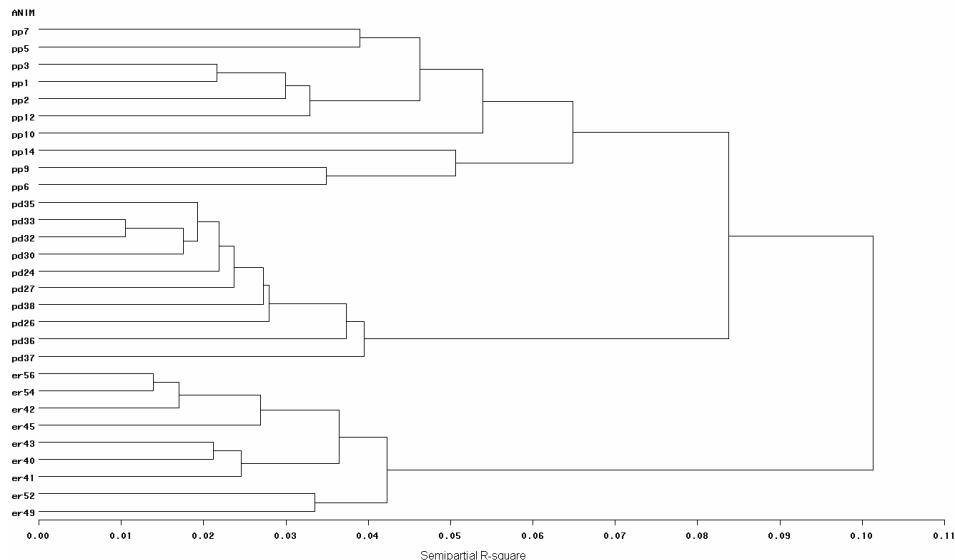
The cluster plot for PP, ER and PD is presented in *Figure 1*. Individual results always well divided into two groups, corresponding to the breeds analysed. Within each sub-cluster, individuals are differently grouped based on similarity on protein expression. The Significance Analysis of Microarrays (SAM) method was adopted to discriminate, among all “statistically” significant spots, those which retain a “biological” significance. This was performed choosing only the spots presenting a volume ratio greater than a predefined Fold Change level. This method was studied to minimize false

positive and to avoid losing information with false negative, especially when few replicates are available. SAM analysis detected 47 differentially expressed significant spots for the confrontations among the breeds, 10 were identified by mass spectrometry. Identified proteins can be divided in two categories: breed specific spots, i.e. spots that are expressed only in a particular breed, and spots that are declared up or down expressed respect to a predefined Fold Change level (fixed to a value of 2). Identified proteins appear heterogeneous in their function. Enzymes, transport, contractile and motile, regulatory and scaffold proteins have been identified and seem hence to play a function in breed differentiation. In particular, Pépoi breed showed two up expressed proteins: GLO1 and HSPB1. GLO1 is a 184 aa long protein of the glyoxalase I family and resulted up expressed in this breed if compared to Ermellinata di Rovigo one. HSPB1, a protein involved in stress resistance and actin organization, although expressed by all analysed breeds, was up-expressed in the Pépoi (FC=4). This result could help in explaining the marked aptitude to environmental adaptation and stress resistance or at least being used to further investigate such characteristic. The Ermellinata di Rovigo breed evidenced two specifically expressed proteins: BRD4 and PGP. They are enzymes respectively involved in the process of cellular mitosis and carbohydrates metabolism, and were not detected in the other analysed breeds. The peculiar expression levels of these proteins could contribute in explaining the differences in terms of growth rates shown by this breed respect to the others. Lastly, the Padovana evidenced two breed specific proteins, CFL2 and ANXA5, and an up expressed protein, APOA1, if compared to both Ermellinata and Pépoi. CFL2 controls actin polymerization and depolymerisation, ANXA 5 is a collagen-binding protein belonging to the annexin family, while APOA1 is a protein participating to the transport of cholesterol from the tissues to the liver.

In general the approach used gave promising results. The method can successfully differentiate the individuals in groups corresponding to the different breeds. It is therefore able to analyze and measure the genetic variation at encoding loci, in a different way to the transcriptomics techniques, which, analyzing the mRNA, are not able to entirely fix the attention on the expressed genes. On the contrary to molecular markers such as SNPs or microsatellites, here the real differences among breeds, due to the different adaptation pressure and selection forces, are outlined. The possibility to isolate and identify the proteins that play the major role in the breed differentiation processes gives the chance to investigate the real biological mechanisms acting at the base. Furthermore, the chicken genome is now sequenced, hence the theoretical identification of all genes product by means of mass spectrometry is now possible. The approach shows however some disadvantages and limitations. The scarce automation makes this method quite laborious and time consuming. Only a part of the entire proteome is easily analyzable by means of this technique, firstly because of its variable nature, that change in response to the external stimuli, secondly because only one tissue among other has been chosen for the analysis, and finally because the fractionation of the protein sample further reduced the number of analyzable proteins. Moreover, the statistical approaches are not yet well developed and most of them are coming from different fields, such as transcriptomics (with whom it shares many important features). Lastly, there is a big interpretative gap from the identification of the most relevant expressed proteins to the explanation of the biological differences mainly because of a lack of knowledge about the function of many proteins. Hence, the detected differences in protein expression, that were here successful in measuring breed differentiation, cannot be used univocally to explain the biological factors involved in this phenomenon.

**Figure 1**

**Ward minimum distance cluster plot for Pèpoi (pp), Padovana (pd) and Ermellinata (er) individuals**



## CONCLUSIONS

The results are promising. This approach can successfully integrate information obtainable with conventional methods for the study of biodiversity. Although this technique does not quantitatively measure the genetic variation within a breed or a population, at least with the tools here used, it succeeds in unfolding the relations among breeds and single individuals. In addition this technique enables to obtain information on the true differences existing at the proteome level among the breeds.

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## **Variation of genetic diversity over time in local Italian chicken breeds undergoing conservation**

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### **ABSTRACT**

*Aim of this study was to measure the variation of different genetic diversity measures in five Italian local chicken breeds over a four years period of conservation. A total of 413 individuals were chosen for the analysis among the animals born in 2002 (a) and in 2006 (b) and genotyped for 20 microsatellite markers. In the animals sampled after four years of conservation activities the loss of alleles occurred, but only for those which had lower frequencies, with a mean number of alleles equal to  $7.75 \pm 3.08$  in the year 2002 and  $4.64 \pm 1.60$  in the year 2006. During the four years of conservation from 2002 to 2006, all breeds showed a not significant decrease of observed heterozygosity, ranging from 2.8% for Robusta Maculata to a maximum of 8.4% for Padovana. Expected heterozygosity showed the same trend, decreasing of 1% for Robusta Lionata to a maximum of 15% for Robusta Maculata. A decrease in the inbreeding coefficient occurred from 2002 to 2006 for Pépoi, Ermellinata di Rovigo and Robusta Maculata breeds, probably due to the unleashing of within breed genetic structures attributable to the rotation of males among the conservation flocks. On the other hand, an increment of the same index was noticed for the Robusta Lionata and Padovana breeds, for which an increase of inbreeding is probably due to the annual selection of individuals for morphological and productive traits. Genetic structure analysis revealed that the best number of populations fitting the dataset was five. Individuals were so assigned to one of five groups, approximately corresponding to the breed of origin. This result shows how no evident genetic structures were detectable within breed, both for 2002 and 2006 individuals. However, an increase in the proportion of membership for each breed has occurred in the year 2006 compared to the data obtained in the year 2002. Results obtained are very useful for planning new strategies for the conservation scheme, including the choice of the animals, a more efficient organization of matings and for the creation of a new selection index based on the maintenance of the existing genetic variation.*

(Keywords: conservation, local chicken breeds, genetic diversity, microsatellites)

### **INTRODUCTION**

Animal biodiversity conservation and management has become an important issue for the international scientific community because of changes in large-scale production systems (FAO, 2007). Therefore, to get information and provide reliable estimation of the genetic diversity within and between a given set of populations the use of molecular markers gained importance, especially in absence of comprehensive breed characterization data and documentation about the origin of breeding populations

(Jordana et al., 2001; Dalvit et al., 2008; Glowatzki- Mullis et al., 2008). Studies about genetic diversity within and between breeds or populations are hence useful to unfold genetic relationships and admixtures and to provide data on evolutionary relationships and parentage within populations (Hillel et al., 2003; Baumung et al., 2004). In the poultry sector a reduction in the number of the local breeds occurred, mainly due to the replacement with cosmopolitan poultry breeds and with highly productive crosses, suggesting an urgent need for conservation of these endangered genetic resources.

In the Veneto region of Italy, ten years ago, local poultry breeds have been put through a governmental *in-situ* marker assisted conservation scheme called “Conservazione e Valorizzazione delle Razze Avicole Venete” (De Marchi et al., 2006). This conservation scheme is based on breed maintenance and multiplication within their production system. Five local chicken breeds were involved, reared in three conservation flocks. The main objectives were to enable the preservation of animal biodiversity and to support the development of marginal areas of the region through the revaluation of these local breeds and their products (De Marchi et al., 2005b). The animals belonging to the conservation programme were investigated and genotyped by the analysis of molecular markers at microsatellite loci. Among all molecular markers, microsatellites were preferred because they are well dispersed in the genome and highly polymorphic (Cheng et al., 1995). They have been used in many countries to study the genetic relationships among local breeds (Baumung et al., 2004; Muchadeyi et al., 2007; Dalvit et al., 2009), and they are suitable for monitoring genetic variability and inbreeding of local chicken breeds involved in the project, so to organize and monitor the conservation activities (Marletta et al., 2006; Dalvit et al., 2008).

Aim of this study was to measure the variation of different genetic diversity measures in five Italian local chicken breeds over a four years period of conservation.

## MATERIALS AND METHODS

The 413 blood samples used for the analysis were chosen among the animals born in 2002 (a) and the ones born in 2006 (b). The breeds involved are five: Ermellinata di Rovigo (ER=45 (a), 45 (b)), Pèpoi (PP=41(a), 45 (b)), Robusta Lionata (RL=26 (a), 43 (b)), Robusta Maculata (RM=45 (a), 45 (b)), and Padovana (PD=28 (a), 50 (b)). Genomic DNA was extracted from blood samples using a modified DNA purification kit (Genta System PUREGENE DNA purification kit). The DNA was used as a template for the PCR reactions. A total of 20 microsatellite loci were investigated (*Table 1*). They are included in the list of recommended markers for chicken of the ISAG/FAO Standing Committee (*MoDAD project, FAO*, 2004). Amplification was performed in multiplex and singleplex reactions at the following conditions: initial denaturation step of 30 s at 98 °C, 40 cycles of 7 s at 98 °C, 15 s at X °C and 20 s at 72 °C, the final extension of 7 min at 72 °C, X° being the annealing temperature for each multiplex and singleplex (available from the National Center for Biotechnology Information). All the details regarding the used protocols are available upon request. Four different poolings were used to analyze the amplified fragments with an automated DNA sequencer (CEQ 8000 Genetic Analysis System, Beckman Coulter, Brea, CA) and the electropherograms were processed with the CEQ 8000 software (Beckman Coulter).

The calculation of the allele frequencies, the estimation of the observed and expected heterozygosity, and the inbreeding coefficient (FIS) were calculated with MolKin (v. 3.0). Test for deviation from Hardy-Weinberg equilibrium was performed by GENEPOP 3.4 (Raymond and Rousset, 1995). Within breed significant differences for

mean number of alleles, observed and expected heterozygosity values calculated for the two years were tested by using PROC UNIVARIATE of SAS (1997). Population structure was inferred by using the software STRUCTURE v. 2.2 (Pritchard *et al.*, 2000). For the analysis a burn-in period of 25.000 iterations and 250.000 repetitions were set, with K ranging from 1 to 15. The best number of clusters fitting the data was established by plotting the mean  $\ln \Pr(X|K)$  for each K., as suggested by Pritchard *et al.* (2000).

## RESULTS AND DISCUSSION

Information about the genetic variability of the twenty investigated loci are listed in *Table 1*. The total number of alleles found was 182 with a mean of  $9.1 \pm 3.67$  and ranging from 3 to 16, for Mcw98 and Mcw104, respectively. The polymorphism information content (PIC) ranged from 0.357 to 0.837, with the minimum value for Mcw103 and the maximum value for the locus Mcw81, with an average of 0.644. Comparing the PIC of all our microsatellite loci used, remarkable differences were not highlighted. In the animals sampled after four years of conservation activities an highly significant ( $P < 0.001$ ) loss of alleles occurred, but only for those which had lower frequencies, with a mean number of alleles equal to  $7.75 \pm 3.08$  in the year 2002 and  $4.64 \pm 1.60$  in the year 2006. For the loci Mcw104, Mcw111 and Mcw16 a loss of 9, 9 and 6 alleles respectively was measured. For this reason the PIC value doesn't differ comparing the two years with a mean of 0.614 and 0.578, for 2002 and 2006 respectively. Causes of this situation are attributable to the impossibility to implement an optimal within family selection, as a group of cocks was mated with a group of hens, so probably not all cocks had the same progeny leading to the loss of some rare alleles. Moreover, the additional selection of individuals on both morphological and productive basis, that is a normal practice for the maintenance of the genetic resources in their productive environment, is also responsible for the loss of alleles.

Wright's fixation indices (FIS, FST, and FIT) for the overall population were 0.096, 0.473 and 0.524 respectively, underlining a high degree of breed differentiation. The genetic variability was studied in terms of Expected (Hexp.) and observed (Hobs.) heterozygosity and inbreeding coefficient (FIS) for each breed analyzed (*Table 2*). In general, heterozygosity estimates were low if compared to other studies (Muchadeyi *et al.*, 2007), but consistent with the data previously reported by Zanetti *et al.* (2010) about these local breeds and similar to those reported by other authors about European pure breeds (Hillel *et al.*, 2003; Granevitze *et al.*, 2007; Dalvit *et al.*, 2009). During the four years of conservation from 2002 to 2006, all breeds showed a decrease of Hobs, ranging from 2.8% for RM to a maximum of 8.4% for PD. Expected heterozygosity showed the same trend, decreasing of 1% for RL to a maximum of 15% for RM. Anyway, within all breeds, significant differences between 2002 and 2006 subjects were not detected for both observed and expected heterozygosity, evidencing that the decrease in heterozygosity values is mainly attributable to the sampling and has a minor impact for the studied breeds. A decrease in the inbreeding coefficient (FIS) occurred from 2002 to 2006 for PP, ER and RM breeds, probably due to the unleashing of within breed genetic structures attributable to the rotation of males among the conservation flocks. On the other hand, an increment of the same index was noticed for the RL and PD breeds, for which an increase of inbreeding is probably due to the annual selection of individuals for morphological and productive traits. New strategy has been adopted recently to contain inbreeding for these two breeds: backcross with the breeds of origin for RL and the

introduction of new unrelated individuals for the PD could succeed in reestablishing contained levels of inbreeding. All the studied breeds showed a persistent significant deviation from Hardy-Weinberg equilibrium at the beginning of the conservation due to a significant excess of homozygotes within breeds, that is maintained after four years, with the only exception of RM that in 2006 reached the equilibrium. The genetic structure of the breeds was analyzed using a Bayesian approach that inferred the number of clusters (K) present in the population, permitting detection of differences among breeds and hidden structures within breeds. *Table 3* describes the proportion of membership of the five local chicken breeds in each of the 5 clusters in the two years (2002 and 2006). The analysis revealed that the best number of populations fitting the dataset was five. Individuals were so assigned to one of five groups, approximately corresponding to the breed of origin. This result shows how no evident genetic structures were detectable within breed, both for 2002 and 2006 individuals. However, an increase in the proportion of membership for each breed has occurred in the year 2006 compared to the data obtained in the year 2002. So within breed individuals in 2006 appear more homogeneous producing clearer, more distinctive and separated groups.

**Table 1**

**Microsatellite markers with corresponding fragment size, chromosomal location, number of alleles and polymorphism information content (PIC)**

Locus	Frag.size (bp)	Chromo-some	Total N. All.	Total PIC	N All. (a)	N. All. (b)	PIC (a)	PIC (b)
Mcw78	134 to 150	5	10	0.659	10	6	0.750	0.571
Mcw104	190 to 228	13	16	0.701	15	6	0.637	0.640
Mcw123	112 to 134	14	12	0.771	6	6	0.561	0.617
Mcw81	143 to 155	5	16	0.837	9	7	0.698	0.718
Mcw14	166 to 189	6	11	0.732	8	3	0.665	0.525
Mcw248	213 to 245	1	10	0.658	8	4	0.371	0.463
Lei94	251 to 283	4	12	0.561	9	7	0.552	0.600
Mcw111	98 to 106	1	13	0.647	13	4	0.699	0.658
Mcw216	141 to 147	13	5	0.547	5	4	0.571	0.602
Mcw222	217 to 225	3	6	0.566	6	4	0.615	0.562
Mcw37	151 to 159	3	5	0.445	4	4	0.493	0.446
Mcw98	255 to 257	4	3	0.428	3	2	0.243	0.166
Adl278	102 to 121	8	8	0.650	6	7	0.682	0.682
Lei166	251 to 261	3	6	0.743	5	3	0.744	0.647
Mcw103	268 to 272	3	4	0.357	4	2	0.479	0.317
Mcw16	136 to 154	3	10	0.713	10	4	0.770	0.675
Mcw165	112 to 123	23	7	0.637	6	4	0.601	0.588
Mcw20	183 to 189	1	9	0.706	9	4	0.659	0.735
Adl268	104 to 119	1	9	0.794	9	6	0.748	0.717
Mcw295	86 to 102	4	10	0.726	10	6	0.744	0.632
Mean			9.10	0.644	7.75	4.65	0.614	0.578

a: sampled in 2002; b: sampled in 2006

**Table 2**

**Expected (Hexp.) and observed (Hobs) heterozygosity, inbreeding coefficient (FIS) for each breed sampled in 2002 (a) and 2006 (b)**

Razze	PP <sup>a</sup> ± SD	PP <sup>b</sup> ± SD	RL <sup>a</sup> ± SD	RL <sup>b</sup> ± SD	ER <sup>a</sup> ± SD	ER <sup>b</sup> ± SD	RM <sup>a</sup> ± SD	RM <sup>b</sup> ± SD	PD <sup>a</sup> ± SD	PD <sup>b</sup> ± SD
Hexp.	0.322 ±0.225	0.228 ±0.236	0.364 ±0.205	0.355 ±0.228	0.553 ±0.179	0.414 ±0.174	0.439 ±0.234	0.289 ±0.222	0.407 ±0.207	0.334 ±0.208
Hobs.	0.278 ±0.254	0.227 ±0.236	0.363 ±0.264	0.311 ±0.264	0.441 ±0.241	0.384 ±0.247	0.320 ±0.262	0.292 ±0.226	0.391 ±0.236	0.306 ±0.225
FIS	0.131	-0.002	0.013	0.121	0.199	0.051	0.281	-0.003	0.042	0.081
P-value	***	*	***	***	***	***	***	N.S.	***	***

ER: Ermellinata di Rovigo; PP: Pépoi; RL: Robusta Lionata; RM: Robusta Maculata; PD: Padovana; SD: standard deviation; <sup>a</sup> 2002; <sup>b</sup> 2006; \* P<0.05; \*\*\* P<0.001; N.S.: not significant

**Table 3**

**Proportion of membership of the local breeds in each of the 5 clusters**

	Clusters									
	2002					2006				
Breed	1	2	3	4	5	1	2	3	4	5
ER	0.007	0.965	0.020	0.002	0.006	0.004	0.982	0.005	0.004	0.005
PP	0.003	0.004	0.006	0.003	0.985	0.005	0.002	0.002	0.003	0.988
RL	0.004	0.039	0.940	0.003	0.013	0.008	0.002	0.985	0.003	0.002
RM	0.961	0.006	0.003	0.026	0.003	0.985	0.002	0.008	0.002	0.003
PD	0.011	0.004	0.006	0.965	0.013	0.003	0.006	0.003	0.985	0.003

## CONCLUSIONS

In conclusion, this research shows the usefulness of molecular markers for monitoring the genetic variability of breeds involved in a conservation scheme. Results obtained are very useful for planning new strategies for the conservation scheme, including the choice of the animals, a more efficient organization of matings and for the creation of a new selection index based on the maintenance of the existing genetic variation. Besides, in order to guarantee higher levels of variability, others approaches should be considered such as sperm cryo-conservation techniques coupled with artificial insemination.

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## Production costs of broiler meat with conventional nutritive composition and with n-3 PUFA enrichment

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### **ABSTRACT**

*Nutritive quality of broiler meat can be influenced through feeding treatments. The research involved 60 Ross 308 broilers divided into 3 groups (C, A and B). In the first three weeks of fattening, broilers were fed starter diets balanced at 24% of crude proteins and 12.5 MJ ME/kg. Experimental period involved last three weeks of fattening. Dietary treatments differed in the source of supplemented oils: C (5% sunflower oil SO), A (2.5% fish oil FO+2.5% sunflower oil SO) and B (2.5% fish oil FO+2.5% linseed oil LO). For the purpose of cost calculation per feeding treatment the following data were considered: average broiler live weight, feed consumption and conversion, and costs of feeding mixtures. Total costs of broiler fattening refer to variable and fixed costs. At the end of fattening period average broiler weights were: A 2836 g, C 2713 g and B 2648 g. Calculation of feed costs per kg of live weight were: C 0.60 €, A 0.58 € and B 0.66 €. Total costs of broiler production per kg of live weight were: C 1.43 €/kg, A 1.38 €/kg and B 1.51 €/kg. It is to conclude that modification of feed composition could reduce the n-6/n-3 PUFA ratio from 10.81% to 1.78% in broiler breasts and from 12.40% to 2.05% in thighs. However, modification of feeding treatments in groups C, A and B affected production costs per one broiler, being 3.88 €, 3.93 € and 4.03 €, respectively.*

(Keywords: broilers, diet composition, fattening costs)

### **INTRODUCTION**

There is a growing consumption of broiler meat not only in Croatia, but also worldwide. This can be explained by broilers' meat satisfactory nutritive and organoleptic traits, as well as by its reasonable pricing. According to Croatian *Statistical Yearbook* (2009), consumption of fresh meat per a household member in Croatia in 2008 was 44.68 kg, of which 16.96 kg was poultry meat. From a nutritive point of view, broiler meat is a dietetic product rich in protein and low in fat (Kralik *et al.*, 2001). Content and profile of fatty acids in poultry meat depend on the composition of fatty acids in poultry diets. Dietary mixtures used for intensive fattening of broilers usually contain high level of saturated fatty acids, and relatively low level of unsaturated fatty acids. Contemporary researches focus on possibilities to modify fatty acid content in poultry products (meat and eggs). Oils originating from sea organisms are known to be rich source of essential n-3 fatty acids (eicosapentaenoic acid-EPA and docosahexaenoic acid-DHA). However, it was proven that those oils added to broiler diets in higher amounts had negative effects on meat organoleptic traits in the sense of „fishy“ odor and taste, which were unacceptable to consumers (Scaife *et al.*, 1994). Linseed and rapeseed oils or seeds can be used as

supplements for broiler diets as an alternative to fish flour or oil (Ajuyah et al., 1991). Significant scientific results are achieved on the respective topic, which resulted in the availability of different animal products labeled as „functional food“ on markets in many countries. Referring to poultry products as functional food, eggs enriched with omega-3 fatty acids are available on Croatian market. This research elaborates the effects of different feeding treatments on the enrichment of meat with n-3 PUFA and on lowering of n-6/n-3 PUFA ratio. Furthermore, following the trends of growing supplies and demands for functional food, this research also presents costs of production of broiler meat with conventional nutritive composition and with enriched n-3 PUFA content.

## MATERIALS AND METHODS

The research was carried out on 60 broilers of Ross 308 provenience. Within the first three weeks of fattening all broilers consumed starter mixture balanced at 24% crude protein and 12.5 MJ ME/kg. The experimental part involved the last three weeks of fattening. After the 21<sup>st</sup> day, broilers were divided into three groups (C, A and B). Group C was given diets that contained 5.0% SO, group A had diets supplemented with 2.5% FO+2.5% SO, and group B had diets with 2.5% FO+2.5% LO. Diets were balanced at 20% crude protein and 13.50 MJ ME/kg. Feeding and watering was *ad libitum* within automatic watering and feeding system. Average weekly weight gains per groups, feed consumption (g) and feed conversion (g/g) were calculated every 7 days. At the end of fattening period, after a 10-hour long starvation, broilers were slaughtered and samples of breasts and thighs were taken for analysis of fatty acid content in muscle lipids. The Chrompack CP-9000 chromatograph equipped with flame ionization detector was used for determination of fatty acids in muscle tissue. Referring to achieved production results and fixed and variable costs of each feeding treatment, the total costs of broiler production were calculated. Research results were processed in Statistica for Windows v.7.1. (StatSoft Inc., 2005). Testing of significance differences between groups was performed by variance analysis (ANOVA). The F-value was compared with theoretic F-value at three significance levels (5% P<0.05; 1% P<0.01 and 0.1% P<0.001). Significance of differences between mean values was determined by the Fischer's LSD-test. The production costs of broiler meat of conventional and modified composition were calculated on the basis of production results and costs of dietary mixtures.

## RESULTS AND DISCUSSION

At the end of fattening period, the highest live weight had broilers of group A, then group C and lastly group B (2830.53 g; 2713.94 g and 2648.31 g, respectively; P>0.05). As shown in the *Table 1*, in the last three fattening weeks group A consumed more feed (4117 g) than groups B and C (3950 g and 3744 g, respectively). Group A achieved the highest weight gain (2032 g), while groups C and B had almost equal gains (1887 g and 1852 g, respectively). The most favorable feed conversion was determined in group C (1.98 g/g), and then in groups A (2.03 g/g) and B (2.13 g/g).

In comparison with group C, presented research results indicated that modified feeding treatments for groups A and B affected the alteration of fatty acid profile in lipids of both breasts and thighs. As seen in *Table 2*, breast of group C contained higher portion of SFA than breasts of groups A and B (P>0.05). Content of SFA in thighs was statistically significantly lower in group A than in groups B and C (25.23%; 27.26% and 27.33%, respectively; P<0.05). Compared to groups C and A, statistically significantly

higher portion of MUFA in broiler breasts and thighs was determined for group B, which was fed diets supplemented with fish and linseed oils ( $P<0.05$ ). As broilers in groups C and A had diets with sunflower oil (5.0% and 2.5%, respectively), the portion of n-6 PUFA in their breasts ( $P<0.001$ ;  $P<0.05$ ) and thighs ( $P<0.001$ ) was statistically significantly higher than of group B, which did not have sunflower oil added to diets.

**Table 1**

**Total consumption, weight gain and feed conversion in the last three weeks of fattening**

Fattening period	Indicators	Groups		
		C	A	B
4 <sup>th</sup> –6 <sup>th</sup> week	Consumption, g	3744	4117	3950
	Weight gain, g	1887	2032	1852
	Feed conversion, g/g of gain	1.98	2.03	2.13

C: 5.0% SO; A: 2.5% FO + 2.5% SO; B: 2.5% FO + 2.5% LO

**Table 2**

**Content of fatty acids in broiler meat (% of total fatty acids)**

Fatty acids	Groups		
	C	A	B
<b>Breasts</b>			
SFA	34.49±4.66	34.64±3.23	32.29±1.98
MUFA	19.12±4.01 <sup>b</sup>	18.46±2.21 <sup>bc</sup>	24.34±1.69 <sup>a</sup>
$\Sigma$ n-6 PUFA	36.23±3.71 <sup>A</sup>	31.67±3.18 <sup>a</sup>	25.62±0.96 <sup>bB</sup>
$\Sigma$ n-3 PUFA	3.35±0.25 <sup>C</sup>	11.11±0.62 <sup>B</sup>	14.44±1.15 <sup>A</sup>
<b>Thighs</b>			
SFA	27.26±1.58 <sup>a</sup>	25.23±1.58 <sup>b</sup>	27.33±1.03 <sup>a</sup>
MUFA	22.91±1.62 <sup>ab</sup>	21.53±2.15 <sup>b</sup>	25.41±1.61 <sup>a</sup>
$\Sigma$ n-6 PUFA	42.92±2.05 <sup>A</sup>	41.09±0.94 <sup>A</sup>	30.91±0.77 <sup>B</sup>
$\Sigma$ n-3 PUFA	3.54±0.17 <sup>C</sup>	11.26±1.01 <sup>B</sup>	15.17±1.28 <sup>A</sup>

<sup>a,b,c</sup>  $P<0.05$ ; <sup>A,B,C</sup>  $P<0.001$

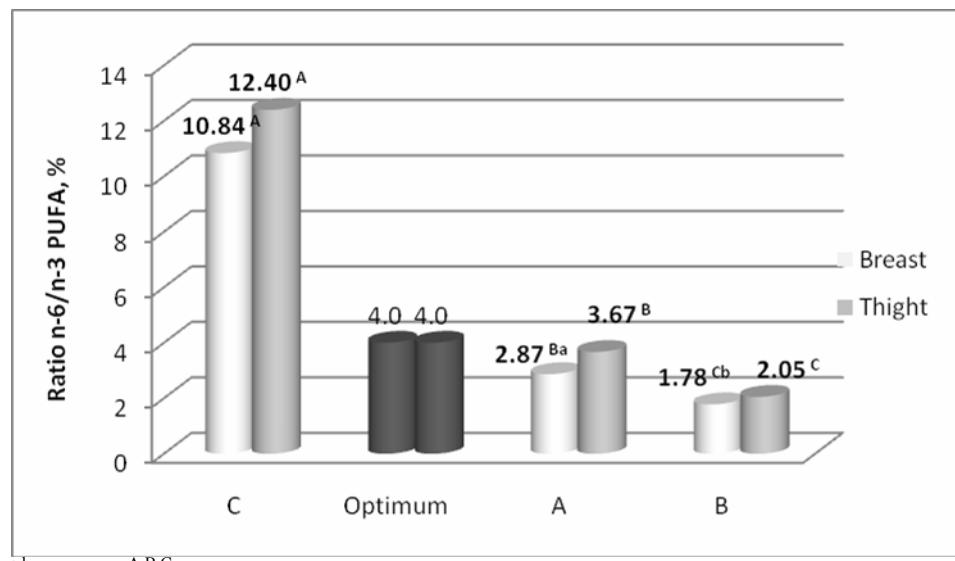
Supplementation of fish oil and linseed oil to diets fed to groups A and B resulted in the increased portion of n-3 PUFA in meat of breasts and thighs. Group B had statistically significantly higher ( $P<0.001$ ) content of n-3 PUFA in breasts and thighs than groups A and C (breasts: 14.44%; 11.11% and 3.35%, respectively; thighs: 15.17%; 11.26% and 3.54%, respectively).

Some scientists stated that people had changed their nutritional habits as they had increased the favorable n-6/n-3 PUFA ratio of 1–4:1 per diet to the high 15–20:1 (Simopoulos, 1998; Sanders, 2000). According to previous results of Kralik *et al.* (2006) referring to n-6/n-3 PUFA ratio of broiler meat available on Croatian market, breast and thigh meat had the ratio of 9.07–11.40:1, and 11.04–15.55:1, respectively. In the present research into effects of broiler diet modification, the n-6/n-3 PUFA ratio was reduced from 10.84% to 1.78% in breast muscles, and from 12.40% to 2.05% in thigh muscles

(Figure 1). Costs of feed per each group (C, A and B), as well as total costs of production per one broiler are presented in *Table 3* and *Table 4*.

**Figure 1**

**The n-6/n-3 PUFA ratio in broiler meat**



<sup>a,b</sup> P<0.01; <sup>A,B,C</sup> P<0.001

**Table 3**

**Costs of diets according to feeding treatments**

Groups	Cost of diets (€/kg)		Diet consumption (kg)		Costs of feed (€/kg)		Broiler weight (kg)
	ST	F1	ST	F2	ST	F3	
C	0.336	0.336	1.090	3.744	0.366	1.25	2.713
A	0.336	0.318	1.090	4.117	0.366	1.31	2.836
B	0.336	0.356	1.090	3.950	0.366	1.41	2.648

ST: starter mixture; F: finisher mixture/1, 2, 3 different groups

As presented in *Table 3*, costs of fattening were equal in all groups for the first three weeks of fattening as all broilers consumed the same starter mixture. Differences with respect to feed costs occurred when broilers started to consume different finisher mixtures. Costs of feed depended on the type of supplemented oil. Finisher diets for groups C, A and B cost 0.336 €/kg, 0.318 €/kg, and 0.356 €/kg, respectively. Costs of feed over three experimental weeks of fattening per kg of live weight depended on the amount of consumed feed and end weight of broilers. Costs per kg of live weight were the lowest for group A, followed by groups C and B (1.38 €/kg, 1.43 €/kg, 1.51 €/kg, respectively). Considering the research objective (enrichment of broiler meat with omega 3 fatty acids), differences in costs per kg of feed were justified.

**Table 4**

**Total cost in broiler production (€/kg of live weight)**

Groups	C	A	B
<b>Fixed costs</b>			
One-day chicken (€/pcs.)	0.68	0.68	0.68
Other costs* (€)	1.57	1.57	1.57
Total fixed costs (€/pcs.)	2.25	2.25	2.25
<b>Variable costs</b>			
Costs of diets (€/pcs.)	1.63	1.67	1.77
<b>Total costs</b>			
Total costs (€/pcs.)	3.88	3.93	4.03
<b>Costs (€/kg of live weight)</b>	1.43	1.38	1.51

\* water, electricity, litter, vaccination, labor

## CONCLUSIONS

On the basis of obtained research results, it can be concluded that supplementation of different oils (sunflower, fish and linseed oil) to broiler diets affected the change of fatty acid profile in broiler meat. Furthermore, modification of broiler diets resulted in lowering of the n-6/n-3 PUFA ratio from 10.84%:1 to 1.78%:1 in breasts, and from 12.40%:1 to 2.05%:1 in thighs. However, it should be emphasized that modification of broiler diets through supplementation of different oils affected the costs per kg of broiler feed and consequently the costs per kg of live weight (C 1.43 €/kg, A 1.38 €/kg and B 1.51 €/kg). But, if considering the production objective, which was to enrich broiler meat with omega 3 fatty acids, differences in costs per kg of feed, as well as in costs per kg of live weight are acceptable and justified.

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## The profit of the slaughterhouses realized in the course of CT-aided selection of rabbits

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### ABSTRACT

*One of the Hungarian rabbit breeding sector's characteristics is that almost all purchased rabbits are exported while their local commerce is minimal. Meat production is one parameter of meat quality which is continuously gaining importance. Improving the meat production of the Pannon White rabbits began in 1992 with the application of Computer Tomography (CT). The objective of the present study was to examine the efficiency of the CT-aided selection from the viewpoint of the slaughterhouses. Analysis of profitability was made using the data of our previous trials taking into account the price obtained for the whole carcass or for the meat fillet. Supposing the same price of Pannon White (P) and Hycole (H) rabbits (trial 1.) marketing the whole carcasses or the meat fillet products resulted in 19 HUF/kg and 43 HUF/kg extra incomes for the Pannon White rabbits. Supposing a slaughter weight of 2.7 kg this value is 51 and 116 HUF per individual. Comparing P×P and P×H genotypes (trial 2) the advantage of the P×P rabbits was 38 HUF/rabbit and 78 HUF/rabbit for whole carcass or the meat fillet product. Comparing the second generation of the divergent selection for thigh muscle volume (trial 3) after 10 generations marketing the whole carcass or the meat fillet product results 68 and 216 HUF extra income per individual (average body weight 2.7 kg) at the slaughterhouse. Supposing the annual slaughter of one million rabbit as a result of the CT-aided selection can increase the profit of the slaughterhouse with 80–100 million HUF. It can be concluded that the selection based on CT data is highly advantageous for the slaughterhouses because they obtain more lean meat from a CT selected rabbit having the same body weight (ie. for which the slaughterhouse paid the same price) which results in a substantial extra income. Thus it would be worth for the slaughterhouses to pay higher price for these animals and share their extra profit with the producer. (Exchange rate between EURO and HUF is about 260–270.)*

(Keywords: rabbit, CT-aided selection, meat production, profit)

### INTRODUCTION

One of the Hungarian rabbit breeding sector's characteristics is that almost all carcasses are exported while their local commerce is minimal. This explains the fact that contrary to our relatively low production Hungary is the largest rabbit exporter in Europe. In Hungary there are six recognized rabbit breeding organizations although most large scale rabbit farms use Hycole hybrids while Pannon White rabbit breed takes the second place. The rabbits produced in Hungary are slaughtered at two abattoirs owned by Olívia Ltd at Lajosmizse and by Tetrabbit Ltd at Baja, respectively (Máté, 2009). Both slaughterhouses process several million rabbits annually thus they have an interest of increasing

meat yield. More than half of the slaughtered rabbits are sold as dissected. Therefore the abattoirs have interest also to increase the percentage and weight of the premium cuts (loin, thigh). Selection for dressing out percentage is not common in rabbit breeding because the trait cannot be measured on live rabbits therefore it could only be determined by progeny test (Varewick et al., 1986; Szendrő et al., 1988). The CT operated at the Kaposvár University makes it possible to determine the meat yield of the rabbits in vivo and to use the data for selection (Romvári et al., 1996). The selection is carried out in two steps. During the first stage the rabbits having the best average daily gains are selected. At the second stage these animals are scanned by CT and only the rabbits having the highest meat yield are chosen for breeding animals.

Between 1992 and 2004 the selection criterion was the cross sectional area of the *M. longissimus dorsi* (at the junction of the 2<sup>nd</sup> and 3<sup>rd</sup> and that of the 4<sup>th</sup> and 5<sup>th</sup> lumbar vertebrae, the so called L-value). The L-value had a moderately high phenotypic correlation ( $r=0.7$ ) with the dressing out percentage (Szendrő et al., 1992). Hoping to increase the efficiency of the selection the selection criterion was changed to the thigh muscle volume in 2005. The CT scans (pictures) were adjusted to take 10 mm thick imaginary slices, from the thigh muscle with total overlapping (slice: 10 mm) providing direct volumetric information. Because the hind legs contain substantially larger amount of muscle than the loin a quicker improvement was expected even in the case of lower efficiency (Szendrő et al., 2008). Nagy et al. (2009) reported a phenotypic correlation of 0.45 between the thigh muscle volume and dressing out percentage. The efficiency of the selection was justified by the annual selection response of 1 g average daily gain and 4 cm<sup>3</sup> thigh muscle volume (Gyovai et al., 2008). The objective of this study was to analyze the usefulness of the selection based on CT data from the viewpoint of slaughterhouses.

## MATERIALS AND METHODS

The profitability analysis of the CT-aided selection (extra income of the slaughterhouse) was made using the data of our previous experiments taking into account the price of the whole carcass and that of the meat fillet. The prices used for calculation were the following: whole carcass 980 HUF/kg, loin fillet (*M. longissimus dorsi*) 3200 HUF/kg, thigh fillet 2100 HUF/kg, carcass without the loin and thigh fillets 450 HUF/kg. Using these prices the income from the slaughtered rabbits were calculated using 1 kg live rabbit as a unit of measurement taking into account whether the rabbit meat was sold as whole carcass or as meat fillet. The calculations were made related to a hypothetical rabbit weighing 2.7 kg and also for 1,000,000 slaughter rabbits. In 2004 Metzger et al. (2006) conducted an experiment comparing Pannon White (P) and Hycole (H) genotypes. The data of the following crossing combinations were compared: P×P, P×H, H×P, H×H. In the experiment carried out in 2007 the following genotypes were used: Pannon White (P), maternal line (A), large sized sire line (N), coloured line (S), Hycole terminal sire line (H). The Pannon White does were inseminated with the spemen of all five genotypes (Szendrő et al., 2010). The slaughter data were evaluated of the following crossing combinations: P×P, P×A, P×H, P×N, P×S. The data of the third trial came from a divergent selection experiment (Szendrő et al., 2008). In this experiment during two generations the Pannon White rabbits were selected with the help of CT to increase and to decrease the thigh muscle volume and the animals showing the highest and lowest values were kept as breeding animals. Experimental data were recorded using Windows Office Excel. The data were evaluated by one factor analysis of variance applying SPSS 10.0 software package. (Exchange rate between EURO and HUF is about 260–270.)

## RESULTS AND DISCUSSION

Slaughter data and the extra income of the first experiment are presented in *Table 1*. From the results the efficiency of the selection is obvious and the superiority of the P×P rabbit can be seen not only from the slaughter data but also from the income compared to other genotypes. According to our data compared to the H×H rabbits the carcass of the P×P and crossed (P×H, H×P) rabbits can be sold obtaining on average 19 and 9 HUF/kg additional income, respectively. In case of marketing meat fillet the extra income of the abattoir for the same genotypes is 43 and 21.5 HUF/kg, respectively, or 116 and 58 HUF/slaughter rabbit weighing 2.7 kg, respectively.

Slaughtering one million rabbits annually the difference between the H×H and P×P genotypes is 116 million HUF which is the additional value of the meat of the selected rabbits.

**Table 1****Profitability analysis of the experiment conducted in 2004**

	Crossing combinations				SE	P
	P×P	P×H	H×P	H×H		
n (1)	60	59	60	52		
Live weight prior to slaughter, g (2)	2644 <sup>a</sup>	2758 <sup>b</sup>	2616 <sup>a</sup>	2671 <sup>a</sup>	12.8	P<0.001
Chilled carcass weight, g	1604 <sup>b</sup>	1652 <sup>c</sup>	1555 <sup>a</sup>	1569 <sup>ab</sup>	7.96	P<0.001
Dressing out percentage, %	60.7 <sup>c</sup>	59.9 <sup>b</sup>	59.5 <sup>b</sup>	58.7 <sup>a</sup>	0.12	P<0.001
Weight of the loin fillet, g	158 <sup>b</sup>	161 <sup>b</sup>	147 <sup>a</sup>	143 <sup>a</sup>	1.29	P<0.001
Loin fillet, % (compared to live weight)	5.97 <sup>c</sup>	5.84 <sup>c</sup>	5.60 <sup>b</sup>	5.34 <sup>a</sup>	0.04	P<0.001
Weight of the thigh fillet, g	365	364	345	342		
Thigh fillet, % (compared to live weight)	13.8 <sup>c</sup>	13.2 <sup>b</sup>	13.2 <sup>b</sup>	12.8 <sup>a</sup>	0.05	P<0.001
Weight of the carcass without the loin and thigh fillets, g	1082 <sup>a</sup>	1127 <sup>b</sup>	1064 <sup>a</sup>	1085 <sup>a</sup>	5.45	P<0.001
Value of the live rabbit HUF (375 HUF/kg)	992 <sup>a</sup>	1034 <sup>b</sup>	981 <sup>a</sup>	1002 <sup>a</sup>	4.80	P<0.001
Value of the chilled carcass, HUF (980 HUF/kg) (3)	1572 <sup>b</sup>	1619 <sup>c</sup>	1524 <sup>a</sup>	1538 <sup>ab</sup>	7.81	P<0.001
Value of the loin fillet, HUF (3200 HUF/kg) (4)	504 <sup>b</sup>	515 <sup>b</sup>	469 <sup>a</sup>	458 <sup>a</sup>	4.14	P<0.001
Value of the thigh fillet, HUF (2100 HUF/kg) (5)	766 <sup>b</sup>	764 <sup>b</sup>	724 <sup>a</sup>	717 <sup>a</sup>	4.43	P<0.001
Value of the carcass without the loin and thigh fillets, HUF (450 HUF/kg) (6)	487 <sup>a</sup>	507 <sup>b</sup>	479 <sup>a</sup>	488 <sup>a</sup>	2.45	
<b>Income related to 1 kg live rabbit, HUF/kg</b>						
Marketing the whole carcass, HUF/kg (3/2)	595 <sup>c</sup>	587 <sup>b</sup>	583 <sup>b</sup>	576 <sup>a</sup>	1.20	P<0.001
Difference compared to the H×H genotype (HUF/kg)	+19	+11	+7	0		
Marketing meat fillet, HUF/kg (4+5+6/2)	665 <sup>c</sup>	648 <sup>b</sup>	639 <sup>b</sup>	622 <sup>a</sup>	2.17	P<0.001
Difference compared to the H×H genotype (HUF/kg)	+43	+26	+17	0		

The results of the experiment conducted in 2007 are provided in *Table 2*. Compared to the P×H group slaughter performance and the income related to 1 kg live weight improved for all crossing combinations. Calculating for the whole carcass the P×N and P×P genotypes realized a higher income by 8 and 14 HUF/kg. In case of meat fillet these differences further increased to 12 and 29 HUF/kg, respectively. Relating these results to a slaughter rabbit weighing 2.7 kg the extra income for these genotypes are 32.4 HUF and 78.3 HUF, respectively which supposing 1,000,000 rabbits leads to 32 and 78 million HUF additional income.

Compared to the first experiment an important difference was that in the second trial in all groups the CT-selected Pannon White does were inseminated with the sperm of other rabbit genotypes that decreased the detectable differences among the groups. It has to be noted that compared to the P×H the large sized sire line selected at Kaposvár University (P×N) gave more favourable results. This genotype is continuously being selected with the use of CT therefore the observed difference might have increased since then.

**Table 2****Profitability analysis of the experiment conducted in 2007**

	Crossing combinations					SE	P
	P×P	P×H	P×N	P×A	P×S		
n (1)	31	28	31	31	30		
Live weight prior to slaughter, g (2)	2659 <sup>a</sup>	2933 <sup>c</sup>	2803 <sup>b</sup>	2701 <sup>ab</sup>	2674 <sup>a</sup>	21.4	<0.001
Chilled carcass weight, g	1602 <sup>a</sup>	1725 <sup>b</sup>	1676 <sup>ab</sup>	1611 <sup>a</sup>	1600 <sup>a</sup>	13.4	0.008
Dressing out percentage, %	60.3	58.9	59.7	59.6	59.8	0.15	0.088
Weight of the loin fillet, g	197	201	198	196	191	2.11	0.658
Loin fillet, % (compared to live weight)	7.41 <sup>c</sup>	6.87 <sup>a</sup>	7.03 <sup>ab</sup>	7.26 <sup>bc</sup>	7.12 <sup>abc</sup>	0.05	0.012
Weight of the thigh fillet, g	382 <sup>ab</sup>	407 <sup>c</sup>	396 <sup>bc</sup>	376 <sup>a</sup>	368 <sup>a</sup>	3.29	0.001
Thigh fillet, % (compared to live weight)	14.4 <sup>c</sup>	13.9 <sup>ab</sup>	14.1 <sup>bc</sup>	13.9 <sup>ab</sup>	13.8 <sup>a</sup>	0.05	<0.001
Weight of the carcass without the loin and thigh fillets, g	1023 <sup>a</sup>	1117 <sup>c</sup>	1082 <sup>bc</sup>	1040 <sup>ab</sup>	1041 <sup>ab</sup>	8.58	0.003
Value of the live rabbit HUF (375 HUF/kg)	997 <sup>a</sup>	1100 <sup>c</sup>	1051 <sup>b</sup>	1013 <sup>ab</sup>	1003 <sup>a</sup>	8.01	<0.001
Value of the chilled carcass, HUF (980 HUF/kg) (3)	1570 <sup>a</sup>	1691 <sup>b</sup>	1642 <sup>ab</sup>	1579 <sup>a</sup>	1568 <sup>a</sup>	13.2	0.008
Value of the loin fillet, HUF (3200 HUF/kg) (4)	631	644	634	627	611	6.77	0.661
Value of the thigh fillet, HUF (2100 HUF/kg) (5)	802 <sup>ab</sup>	855 <sup>c</sup>	832 <sup>bc</sup>	789 <sup>a</sup>	773 <sup>a</sup>	6.90	0.001
Value of the carcass without the loin and thigh fillets, HUF (450 HUF/kg) (6)	460 <sup>a</sup>	503 <sup>c</sup>	487 <sup>bc</sup>	468 <sup>ab</sup>	468 <sup>ab</sup>	3.86	0.003
<b>Income related to 1 kg live rabbit, HUF/kg</b>							
Marketing the whole carcass, HUF/kg (3/2)	591	577	585	584	586	1.49	0.085
Difference compared to the P×H-genotype (HUF/kg)	+14	-	+8	+7	+9		
Marketing meat fillet, HUF/kg (4+5+6/2)	712 <sup>b</sup>	683 <sup>a</sup>	695 <sup>a</sup>	698 <sup>ab</sup>	692 <sup>a</sup>	2.50	0.006
Difference compared to the P×H-genotype (HUF/kg)	+29	-	+12	+15	+9		

The divergent selection for thigh muscle volume resulted a 1.1% difference in the dressing out percentage and 1.2% difference in the thigh muscle fillet compared to the live weight (*Table 3*). The difference between the groups was 10 and 32 HUF/kg for the whole carcass and meat fillet, respectively (*Table 3*). This is equivalent of an additional income of 2.5 and 8.0 HUF/kg per generation for whole carcass and for meat fillet, respectively. Supposing the same selection response after 10 generations the slaughterhouses can realize an additional income of 25 and 80 HUF, respectively. Considering 2.7 kg slaughter weight these values are 67.5 and 216 HUF/rabbit. Supposing 1,000,000 slaughtered rabbits annually the extra profit of the slaughterhouse are 67.5 and 216 million HUF. Because the Pannon White and the large size sire line rabbits are selected with the aid of the CT since 1992 and 2005, respectively, the ten generation long interval cannot be considered to be exaggerated.

**Table 3**

**Profitability analysis of the divergent selection experiment**

	Divergent selection		SE	P
	Negative	Positive		
n (1)	24	24		
Live weight prior to slaughter, g (2)	2454	2445	29.6	0.881
Chilled carcass weight, g	1444	1462	21.2	0.682
Dressing out percentage, %	58,7	59,8	0.30	0.086
Weight of the loin fillet, g	137	142	2.69	0.358
Loin fillet, % (compared to live weight)	5,56	5,81	0.08	0.135
Weight of the thigh fillet, g	327	355	6.15	0.019
Thigh fillet, % (compared to live weight)	13,3	14,5	0.16	<0.001
Weight of the carcass without the loin and thigh fillets, g	981	965	13.7	0.570
Value of the live rabbit HUF (375 HUF/kg)	920	917	11.1	0.881
Value of the chilled carcass, HUF (980 HUF/kg) (3)	1415	1432	20.7	0.684
Value of the loin fillet, HUF (3200 HUF/kg) (4)	438	454	8.61	0.354
Value of the thigh fillet, HUF (2100 HUF/kg) (5)	686	745	12.9	0.019
Value of the carcass without the loin and thigh fillets, HUF (450 HUF/kg) (6)	441	434	6.16	0.570
<b>Income related to 1 kg live rabbit, HUF/kg</b>				
Marketing the whole carcass, HUF/kg (3/2)	576	586	2.98	0.088
Difference compared to the MM-genotype (HUF/kg)	-	+10		
Marketing meat fillet, HUF/kg (4+5+6/2)	636	668	5.55	0.003
Difference compared to the MM-genotype (HUF/kg)	-	+32		

**CONCLUSIONS**

The selection based on CT data is highly advantageous for the slaughterhouses because they obtain more lean meat from a CT selected rabbit having the same body weight (ie. for which the slaughterhouse paid the same price) which may result 100 million HUF extra income. Thus it would be worth for the slaughterhouses to pay higher price for these animals and share their extra profit with the producer to induce the use of Pannon White rabbits and their crosses. Subsidizing the CT investigation would quickly return as

the progeny of the selected rabbits (having higher meat yield) would be transported to slaughterhouses. Selection for meat production is also advantageous for the producer as these rabbits have better feed conversion ratio.

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## **SECTION 3**

### **CATTLE BREEDING**





## Concentration of noxious gases in dairy, beef and veal calves farms in Northern Italy

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### ABSTRACT

*Aim of this paper was investigating carbon dioxide and ammonia concentrations through a cross-sectional study applied during winter 2007 in 35 dairy cattle, 21 beef cattle and 30 veal calves farms located in North-eastern Italy. Repeated instrumental gas measurements were carried out in the feeding alley and in the resting area. Regardless of the measure point, gases concentrations in the three categories of cattle did not give reason for concern since average values were below the threshold limit set for animals. Significant difference ( $P<0.05$ ) were observed among cattle categories with the worse air quality detected in veal calves farms. Twenty percent of these farms had at least one ammonia measure exceeding the recommended level of 10 ppm. Carbon dioxide was higher ( $P<0.001$ ) in closed barns compared to open barns for both, dairy and beef cattle indicating a worse ventilation efficiency. Within closed barns tethered compared to loosed housed cows showed higher carbon dioxide concentrations. Similar results were found for beef cattle on fully slatted floors compared to bulls on deep litter. These results should drive attention towards specific management practices and facilities but, in order to obtain a full picture on the levels of gases concentration in cattle farms, further research should consider their emissions in different seasons as well as cattle age, breed, and productive level.*

(Keywords: dairy cows, beef cattle, veal calves, greenhouse gases, ammonia)

### INTRODUCTION

In the last decades, several studies demonstrated the importance of the farm microclimatic conditions for animal welfare. Continuous intake and oxygen/carbon dioxide exchange between the animal organism and the environment makes the respiratory system constantly exposed to the farm micro-climatic conditions. Particular attention was therefore given to the presence of noxious gases concentrations that are considered harmful for health of both, farmers and animals. The recent EFSA (2009) on the effects of farming systems on dairy cow welfare and disease, indeed, reports ammonia, methane and carbon dioxide as some of the most relevant gases.

Ammonia is an irritant gas that originates from chemical/biological breakdown of livestock urine and faeces (Groot Koerkamp *et al.*, 1998). High concentrations of ammonia cause inflammation of the respiratory and ocular mucosa and could be noxious for integumental and neuronal systems. In regards to the respiratory system, prolonged exposure to ammonia, decreases the number of ciliated epithelial cells, and consequently acts as a predisposing factor for the development or for enhancing severity of respiratory disorders (Marschang, 1973; Wathes, 1994; Hartung, 1994). In some extreme cases ammonia may even be fatal.

Carbon dioxide is a trace gas currently composing about 0.038% of the global atmosphere equivalent to about 380 ppm by volume (IPCC, 2007). It is a greenhouse gas and its concentration varies seasonally and on a regional basis since in urban areas concentrations are generally higher. Indoors carbon dioxide concentration can reach levels up to ten times higher than environmental once. This confirms that carbon dioxide is a very useful indicator for the assessment of air quality and of ventilation intensity in the case of farms micro-climate conditions (EFSA, 2006).

Since intensive rearing conditions could be considered borderline between physiological and pathological, fingers are often pointed out on this farming system from the welfare point of view. Several actions have been done in order to set or recommend acceptable levels that guarantee animal health and welfare in Europe. According to the EFSA (2009) report, the threshold limit for carbon dioxide in animal house atmosphere is 3000 ppm, while for ammonia it is set to 20 ppm. However, according to the Scientific Committee on Animal Health and Animal Welfare (SCAHAW, 2001) and the Swedish animal welfare legislation (Lundborg et al., 2005) ammonia should not exceed 10 ppm for cattle.

It was aim of the current study, therefore, to investigate on-field concentrations of the potentially noxious gases in dairy and beef cattle and veal calves farms at the existing farming conditions.

## MATERIALS AND METHODS

The cross-sectional study was carried out during winter 2007 on a sample of 86 commercial farms located in the Veneto region (North-East Italy). All farms were specialized units: 35 rearing dairy cows, 21 rearing beef cattle and 30 rearing veal calves. Every farm was visited ones by a trained assessor who gathered information regarding the housing system and measured gases concentrations.

Instrumental gas measurements were carried out adopting the Dräger X-am 7000 device (Dräger Safety Italia S.p.A., Milano, Italy) that detects methane, carbon dioxide, ammonia, hydrogen sulphide and sulphur dioxide and is provided with a telescopic probe. This allowed the assessor to remain in the feeding alley avoiding also excessive animals movements. Considering that spatial distribution of gases concentrations differ inside a single animal barn three repeated measurements were taken at a standardized distance from the animals both, in the feeding alley and in the resting area.

At the time of the gases concentration measurements, micro- and macro-climate temperature was also assessed using a termohygrometer (Delta OHM S.r.l., Padova, Italy).

Data were submitted to statistical analysis with the SAS package (SAS, 2003). Farm distribution and frequency were studied using the PROC FREQ procedure. Analysis of variance was carried out adopting a mixed model approach with the PROC MIXED statement considering the main effect as fixed and the farm and repeated measurement effects as random. Main effects studied were at first the category of cattle (dairy, beef and veal calves), the analysis was later on restricted to open versus closed barns in dairy and beef cattle farms and to the type of housing system or floor where present such distinction. Results were considered statistically significant for  $P < 0.05$ .

## RESULTS AND DISCUSSION

Farm sample distribution according to the housing conditions showed that, among the 35 dairy farms, 23 reared cows in a loose housing system with the adoption of cubicles

either in open or closed barns while the remaining farms reared cows tethered in close barns. Beef cattle were fattened on fully slatted floors in 11 farms and on deep litter in 10 farms. All veal calves were reared indoors on fully slatted floors either of concrete (12 farms) or wooden (18 farms).

The present study focused mainly on the concentrations of carbon dioxide and ammonia while methane, hydrogen sulphide and sulphur dioxide were omitted from results. Methane was not detected in any farm and the remaining 2 gases were detected only in veal calves farms in negligible concentrations. Descriptive statistics regarding environmental temperature showed that on average it remained within seasonal ranges and, considering that the study was carried out during winter, temperature was not further on included in the analysis.

As shown in *Table 1* regardless of the measure point, carbon dioxide and ammonia concentrations were below the threshold limit set for animals (EFSA, 2009) in all the three categories of cattle. However, the significant differences among cattle category underlined that veal calves farms had the highest gases concentrations (*Table 1*). In regards to carbon dioxide it is interesting to notice that the minimum recorded concentrations are similar to the values reported for the composition of the global atmosphere (IPCC, 2007), indicating likely good air exchange in these farms.

Positive results were also found looking at the concentrations of ammonia since they never reached 4 ppm (*Table 1*). Values detected in this study confirm results reported by *Groot Koerkamp et al.* (1998) for the three considered categories of cattle. Similarly to carbon dioxide, the highest ammonia concentrations were recorded in veal calves barns where the maximum value reached 15 ppm, comparable to the level recorded in group housed calves on slats in the Netherlands (*Groot Koerkamp et al.*, 1998). A detailed picture of the farm distribution according to the threshold ammonia level of 10 ppm recommended for cattle by the Scientific Committee on Animal Health and Animal Welfare (SCAHAW, 2001) and accepted by the Swedish animal welfare legislation (*Lundborg et al.*, 2005) showed, indeed, that 20% of the veal calves farms had at least one ammonia measure above such value. Poor air quality detected in this study for veal calves is in accordance with the main characteristics of the standardized veal calves production system that is based on indoor housing in closed barns on fully slatted floors (*Cozzi et al.*, 2009).

**Table 1**

**Concentration of carbon dioxide and ammonia detected in the feeding alley and in the resting area in dairy, beef and veal calves farms**

Item	Dairy cattle farms (n=35)		Beef cattle farms (n=21)		Veal calves farms (n=30)		RMSE
	Lsmean	Min-Max	Lsmean	Min-Max	Lsmean	Min-Max	
<i>Carbon dioxide (ppm)</i>							
feeding alley	703 <sup>b</sup>	300–2000	609 <sup>c</sup>	300–1100	861 <sup>a</sup>	300–2200	260.3
resting area	781 <sup>b</sup>	300–1600	770 <sup>b</sup>	400–1200	977 <sup>a</sup>	400–2200	257.5
<i>Ammonia (ppm)</i>							
feeding alley	0.3 <sup>b</sup>	0–6	0.0 <sup>b</sup>	0–0	2.9 <sup>a</sup>	0–15	2.16
resting area	0.9 <sup>b</sup>	0–11	0.4 <sup>b</sup>	0–8	3.7 <sup>a</sup>	0–15	2.75

Different superscript letters within row mean significantly different for P<0.001

Unlikely from the standardized veal calves production, dairy and beef cattle could be reared adopting different housing systems. A first distinction between open and closed barns allowed us to assess differences in gases concentrations for dairy and beef cattle reared in the two types of barns (*Table 2*). As expected, open barns showed lower carbon dioxide concentrations in both categories of cattle either when detected in feeding alley or in the resting area. Ammonia concentration was significantly lower in the feeding alley of open dairy farms while it showed no difference in the other cases.

**Table 2**

**Carbon dioxide and ammonia concentrations detected in open versus closed barns in dairy and beef cattle farms**

Item	Dairy cattle farms				Beef cattle farms			
	Open barn (n=18)	Closed barn (n=17)	SE	P	Open barn (n=5)	Closed barn (n=16)	SE	P
<i>Carbon dioxide (ppm)</i>								
feeding alley	519	898	43.4	***	467	654	39.7	***
resting area	593	980	39.4	***	640	810	44.2	***
<i>Ammonia (ppm)</i>								
feeding alley	0.0	0.59	0.20	**	0.0	0.0	-	-
resting area	0.63	1.14	0.41	ns	0.0	0.5	0.36	ns

ns: not significantly different; \*\* significantly different for  $P<0.01$ ; \*\*\* significantly different for  $P<0.001$  within cattle category

Since carbon dioxide was higher in closed barns, a second step was the investigation, within cattle category, of the housing system that has detrimental effects on air quality. Looking at results in *Table 3*, it is noteworthy that carbon dioxide was more concentrated in tie dairy farms regardless of the place of measurement. Ammonia concentrations were not significantly different, but the lower level recorded in the resting area for tethered cows is in accordance with the trend reported by *Amon et al.* (2001) who assumed a likely higher ammonia emission in loose housing systems due to their different design.

**Table 3**

**Carbon dioxide and ammonia concentrations detected in closed barns according to the housing system in dairy cattle farms and to the type of floor in beef cattle farms**

Item	Dairy cattle farms				Beef cattle farms			
	Loose housing (n=5)	Tie stall (n=12)	SE	P	Slatted floor (n=9)	Deep litter (n=7)	SE	P
<i>Carbon dioxide (ppm)</i>								
feeding alley	687	986	80.6	***	707	586	37.6	**
resting area	793	1058	62.7	***	863	743	43.5	**
<i>Ammonia (ppm)</i>								
feeding alley	0.0	0.8	0.45	ns	0.0	0.0	-	-
resting area	1.5	1.0	0.57	ns	0.6	0.4	0.41	ns

See *Table 2*.

In beef cattle farms, slatted floors seem worsening air quality since both higher carbon dioxide and ammonia concentrations were detected even though ammonia levels were not significantly different (*Table 3*). The same trend was recorded by *Groot Koerkamp et al.* (1998) who studied ammonia concentrations and emissions in different livestock categories. It is likely that different manure handling affects gas emission in the farms, and in particular when liquid manure is kept underneath fully slatted pens (*Cozzi et al.*, 2009).

## CONCLUSIONS

Actual levels of carbon dioxide and ammonia were quantified through a cross-sectional study carried out on a sample of dairy, beef and veal calves farms in North-eastern Italy during winter. In comparison to other livestock categories such as poultry and pigs, results obtained for cattle showed that there is no reason for concern since the gases concentrations were always below the threshold limits. However, poor air quality recorded in some farms rearing veal calves in particular should drive attention towards specific management practices and facilities in order to improve it. Implementation of suitable barn design, appropriate ventilation and apt manure storage could be some practical examples.

In order to obtain a full picture on the levels of gases concentration in cattle farms, further research should, however, consider gases emissions in different seasons as well as cattle age, breed, and productive level.

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## The effect of rearing technology on carcass quality of Cika young bulls

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### **ABSTRACT**

*Cika cattle is the only Slovenian autochthonous cattle breed. Nowadays, it is mostly reared in the cow-calf systems. The aim of this study was to find out how rearing technology affects carcass and beef quality of slaughtered Cika bulls. This study included 18 young bulls of Cika cattle; 8 bulls were intensively fattened, while 10 young bulls were grazing on the pastures. Data were analysed by Student t-test using PROC TTEST in the statistical package SAS/STAT considering rearing technology. Young bulls reared on pasture until slaughter had lower carcass weights (232.8 kg vs. 291.8 kg), they were older at slaughter (23.54 months vs. 19.99 months) and reached lower net daily gains (330.5 g/day vs. 488.4 g/day) than fattened young Cika bulls. Average conformation score in carcasses of fattened were significantly higher (7.13) compared to grazed bulls (5.20) as well as fatness score was significantly higher in carcasses of fattened (5.38) compared to grazed bulls (3.40). In carcass sides lean meat had largest part, but the difference between fattened (73.07%) and grazed bulls (73.79%) was not significant. Side carcasses of fattened bulls had significantly larger part of fat (8.91%) compared to 5.95% of fat in carcasses of grazed bulls. The lean meat: bones ratio in fattened carcasses was significantly higher (4.52) compared to grazed carcasses (4.08). Grazed bulls had significantly larger lean meat: fat ratio (14.19) compared to fattened bulls (8.30).*

(Keywords: cattle, Cika cattle, autochthonous breed, rearing technology, carcass quality)

### **INTRODUCTION**

Cika cattle is the only Slovenian autochthonous cattle breed. In 2009, the population of Cika cattle is numbering 2159 animals (Sector for Identification and Registration at the Ministry for Agriculture, Forests and Food). Breeding goal for Cika cattle is dual purpose breed with the emphasis on milk production. Cika cattle were traditionally kept for milk production. However, to a smaller extent it is still used for milk production in traditional regions of Alpine dairy-farming. Nowadays, Cika is mostly reared in the cow-calf systems for beef production only, which is not in accordance with the breeding goal. Simčič *et al.* (2008) reported of carcass quality traits of all categories of Cika cattle from data collected in the slaughterhouses in years 2005 to 2007. There were no known data about rearing technology and how it affects the carcass and beef quality of Cika cattle. It is well known that rearing technology also affects the consumer's preferences of choosing beef. This preference includes both, the ecological as well as ethological aspects. The aim of this study was to find out how rearing technology (intensive fattening vs. traditional grazing) affects some traits of carcass and beef quality of slaughtered young Cika bulls.

## MATERIALS AND METHODS

This study included 18 young bulls of the autochthonous Cika cattle, 8 bulls were intensively fattened at the Educational and Research Animal Husbandry Centre Logatec (ERC) (Slovenia), while 10 young bulls were grazing on the pastures at four breeders throughout Slovenia. Intensively fattened bulls were moved in the ERC from various suckler herds after weaning at the average age of 233.3 days and average body weight 247.8 kg. Fattened bulls were fed with total mix of maize and grass silage and concentrates. For the first year grazed bulls were in suckler herds together with their mothers till weaning. Bulls were housed during the winter period. In the spring they were put on all day grazing with no added concentrates on the pastures in four different places in Slovenia. Fattened bulls at ERC were slaughtered when they achieved subjectively evaluated optimal fatness. Grazed young bulls were traditionally moved to the slaughterhouses when the vegetation period finished. The average slaughter weight of fattened bulls was 543.6 kg, while slaughter weight of grazed bulls was unknown, because breeders did not weigh their bulls prior to the slaughter. Slaughter age was 20.0 months in average of fattened and 23.5 months of grazed bulls. The average daily gain from birth to slaughter calculated from the body weight and slaughter age was 910.6 g/day at fattened young bulls. After the slaughter, hot carcass weight, carcass length and chest depth were recorded. Carcass length was measured as the distance from the front edge of the pelvic symphysis to the middle of front edge of the first rib. Chest depth is the distance from the ventral edge of the spinal canal to the ventral edge of the broken sternum of the fifth rib. Carcass conformation and fatness were scored according to the EUROP system (Čepon et al., 2006). Net daily gain was calculated from hot carcass weight and age at slaughter. Index of conformation was computed as quotient between hot carcass weight and the product of carcass length and chest depth. The pH<sub>48</sub> was measured 48 h after slaughter in the *Longissimus dorsi* muscle behind the last rib using a pH-meter equipped with a penetrating electrode. Beef colour was measured as triplicate on the cross section of *Longissimus dorsi* muscle after 30 min of exposure to the air by chromo meter (Minolta CR 300) and expressed as CIE L\*a\*b\* values. After chilling the right carcass side of each carcass was separated into the main carcass tissues (lean meat, fat, tendons, bones). The total weight of separated tissues was used to calculate the proportions of four various tissues in the carcass. Data were analysed by Student t-test using PROC TTEST in the statistical package SAS/STAT (SAS Institute Inc., 2001) considering rearing technology.

## RESULTS AND DISCUSSION

Several studies have been reported to characterize carcass traits of young bulls belonging to the some European beef and dual purpose breeds, as well as autochthonous breeds (Albertí et al., 2008). Some of them considered typical production systems for fattening young bulls of autochthonous breeds (Piedrafita et al., 2003; Serra et al., 2004), while others tried to intensively fatten young bulls of autochthonous breeds to show their potential for beef production (Cozzi et al., 2009; Özlıtürk et al., 2004). In this study a traditionally grazed vs. intensively fattened autochthonous Cika young bulls were included.

Grazed young bulls (23.54 months, 706 days) were in average significantly older at slaughter compared to intensively fattened bulls (19.99 months; 600 days) (Table 1). In average hot carcass weight of fattened bulls was significantly higher (291.8 kg)

compared to (232.8 kg) carcasses of grazed bulls. Standard deviations of hot carcass weight were large among grazed as well as fattened bulls. In previous study, *Simčič et al.* (2008) investigated carcass traits of 249 Cika bulls in the A category (bulls under 24 months). Young bulls originated from various farms throughout Slovenia and different rearing technologies. Bulls were slaughtered in abattoirs, where data were collected. However, carcass weight was 260.3 kg, which is less than carcass of fattened bulls (291.8 kg) and more than carcasses of grazed bulls (232.8 kg) in this study. The average daily gain as well as dressing percentage was not computed, because the slaughter weight was unknown in the group of grazed bulls. Bulls in the previous study (*Simčič et al.*, 2008) were slaughtered at an average age of 18.6 months and achieved 474.3 g/day net daily gain. The only computed gain in both groups of bulls in this study was net daily gain, which was significantly higher in fattened (488.4 g/day) compare to grazed bulls (330.5 g/day). It should be considered that grazed bulls have not been supplemented with concentrates.

**Table 1**

**Age at slaughter, hot carcass weight, net daily gain and conformation and fatness scores of Cika young bulls**

Trait	Fattened bulls (n=8)	Grazed bulls (n=10)	P-values
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	
Age at slaughter (months)	19.99 $\pm$ 1.69	23.54 $\pm$ 0.74	<0.0001
Hot carcass weight (kg)	291.8 $\pm$ 39.0	232.8 $\pm$ 48.9	0.0136
Net daily gain (g/day)	488.4 $\pm$ 65.9	330.5 $\pm$ 74.0	0.0002
Conformation (EUROP)*	7.13 $\pm$ 1.55	5.20 $\pm$ 0.63	0.0025
Fatness (1–15)	5.38 $\pm$ 1.41	3.40 $\pm$ 1.26	0.0064
Carcass length (cm)	129.3 $\pm$ 3.67	127.5 $\pm$ 6.32	n.s.
Chest depth (cm)	42.36 $\pm$ 1.62	40.77 $\pm$ 1.90	n.s.
Index of conformation	53.09 $\pm$ 4.75	44.38 $\pm$ 6.18	0.0048

\* (E+=15, E+=14, E-=13, U+=12, U+=11, U-=10, R+=9, R+=8, R-=7, O+=6, O+=5, O-=4, P+=3, P+=2, P-=1); SD: standard deviation

Very close slaughter age to grazed Cika bulls (706 days) found *Piedrafita et al.* (2003) in Aubrac bulls (723 days) which were grazed and in the end of fattening period were fed with maize silage, hay and concentrates. Salers bulls were at slaughter 582 day old, which is 20 days less than fattened Cika bulls in this study. Salers bulls were fattened with grass and maize silage, and completed with concentrates. Both, Aubrac and Salers breeds in France were originally used for draught and milk production. Nowadays, they produce purebred and crossbreed weaned calves for fattening (*Piedrafita et al.*, 2003).

*Cozzi et al.* (2009) found that Burlina bulls, a native dual purpose breed in Italy, experimentally fattened, achieved very similar slaughter weight (546.0 kg) and hot carcass weight (290.9 kg) as fattened Cika bulls (543.6 kg, 291.8 kg), respectively. However, Burlina young bulls needed 126 days less fattened period.

Carcass conformation and fatness were scored according to the EUROP system with 15 possible scores (*Čepon et al.*, 2006). The average conformation score in carcasses of fattened bulls were significantly higher (7.13) compared to grazed bulls (5.20) as well as fatness score was significantly higher in carcasses of fattened (5.38)

compared to grazed bulls (3.40). Index of conformation computed from hot carcass weight, carcass length and chest depth were also significantly higher in carcasses of fattened (53.09) compared to grazed bulls (44.38). Grazed bulls had “thinner” carcasses. There were no significant differences in carcass length and chest depth between fattened and grazed bulls (*Table 1*). Carcass sides of fattened bulls were in average longer and deeper than carcass sides of grazed bulls.

Similar average carcass conformation (7.5) scored by EUROP classification have been found at young bulls of the Asturiana de la Montaña small to medium-sized local cattle breed in Spain, compared to fattened Cika bulls (7.13). The average fatness score (5.5) in Pirenaica, the Spanish medium to large-sized local cattle breed, was also similar to fattened Cika bulls (5.38). Young bulls of the Spanish local breeds were fattened at about 5–8 months of age with concentrated meal and straw or hay (*Piedrafita et al., 2003*).

In carcass sides lean meat presented the largest part, but the difference between fattened (73.07%) and grazed bulls (73.79%) was not significant. Side carcasses of fattened bulls had significantly larger part of fat (8.91%) compared to 5.95% of fat in carcasses of grazed bulls. However, a larger part of bones (18.20%) and tendons (2.06%) was found in carcasses of grazed bulls compared to fattened bulls (16.22%, 1.80%), respectively (*Table 2*). In this study, the lean meat: bones ratio in fattened carcasses was significantly higher (4.52) compared to grazed carcasses (4.08). However, grazed bulls had significantly larger lean meat: fat ratio (14.19) compared to fattened bulls (8.30).

*Albertí et al. (2008)* reported of very similar lean meat (72.9%), fat (9.7%), and bones (17.5%) proportion in carcasses of the Spanish local breed Pirenaica compared to fattened Cika bulls (73.07%, 8.91%, 16.22%), respectively. Young bulls of Pirenaica were fattened with concentrated meal and straw or hay.

Similar proportion of lean meat and bones was found also in fattened young bulls of the local breeds in France, Gascone and Salers. Gascone carcasses had 73.9% lean meat and 14.5% bones, while Salers carcasses had 73.4% lean meat and 15.2% bones (*Piedrafita et al., 2003*).

**Table 2**

**Carcass composition and proportions of Cika young bulls**

Carcass composition	Fattened bulls (n=8)	Grazed bulls (n=10)	P-values
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	
Lean meat (%)	73.07 ± 1.03	73.79±2.15	n.s.
Fat (%)	8.91 ± 1.00	5.95±2.07	0.0020
Bones (%)	16.22 ± 0.88	18.20±1.52	0.0050
Tendons (%)	1.80 ± 0.23	2.06±0.16	0.0115
Lean meat:bones ratio	4.52 ± 0.28	4.08±0.40	0.0195
Lean meat:fat ratio	8.30 ± 1.02	14.19±6.03	0.0151

pH and beef colour of *M. longissimus dorsi* are presented in *Table 3*. Differences in pH value and beef colour (CIE L, a, b) of two groups of young bulls were not significant. Even known that beef become darker with age, the older grazed Cika bulls in this study had tendency to have lighter beef than younger fattened ones.

The colour of beef in *M. longissimus dorsi* of Brown young bulls and Black and White young bulls fattened in the similar conditions (ERC) as Cika bulls were reported by *Čepin et al. (2001)*. Black and White bulls (L\*=36.99, a\*=21.33, b\*=10.71) as well

as Brown bulls ( $L^*=36.69$ ,  $a^*=21.48$ ,  $b^*=10.56$ ) had lighter and less red beef compared to fattened Cika young bulls.

**Table 3**

**pH value and beef colour of *M. longissimus dorsi* of Cika young bulls**

<b>Trait</b>	<b>Fattened bulls (n=8)</b>	<b>Grazed bulls (n=10)</b>	<b>P-values</b>
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	
pH (48 h after slaughter)	5.72±0.09	5.69±0.11	n.s.
CIE L*	36.15±1.89	36.59±1.42	n.s.
CIE a*	25.19±3.47	24.42±1.94	n.s.
CIE b*	12.79±1.91	12.84±1.22	n.s.

## CONCLUSIONS

Rearing technology (intensive fattening *vs.* traditional grazing) significantly affected carcass traits of young bulls of Cika cattle. Young bulls reared on pasture until slaughter had lower carcass weights, they were older at slaughter, reached lower net daily gains and lower conformation and fatness scores. Grazed bulls had in carcasses almost equal proportion of lean meat, lower proportion of fat and higher proportion of bones compared to fattened bulls. There were no significant differences in pH<sub>48</sub> and beef colour between grazed and fattened young Cika bulls. Grazed bulls were slaughtered in the end of vegetation period without supplemented with concentrates. It can be recommended to fatten young bulls of Cika cattle after the end of the vegetation period to achieve larger slaughter weights and hot carcass weights, as well as better conformation and fatness scores.

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## Effect of Brown cattle crossing with beef breeds on growth and carcass traits

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### ABSTRACT

*The aim of the study was to investigate the effect of crossing Brown breed dams with beef breeds sires (Charolais, Limousine and Belgian Blue) on growth and carcass performances of their offspring. Bulls younger than 24 months, bulls older than 24 months and calves slaughtered in Slovenian slaughterhouses from January 2007 to December 2009 were included in the comparison. In all three categories all crossbreds had heavier carcass and better net daily gain than pure breed Brown animals. The highest carcass weight and net daily gain were achieved by Charolais crossbred bulls. On average, the conformation of crossbred bulls was for more than two subclasses better than that of Brown bulls, where the Belgian Blue crossbred bulls reached the best conformation score. Carcass fatness varied from 5.7 to 6.7 (fatness class between 2+ and 3-) among all bulls' genotypes and was the lowest in Belgian Blue crossbreds. Crossbred calves were heavier with better net daily gain and were scored for 2 conformation subclasses better than Brown breed calves. Again the highest net daily gain was achieved by Charolais crossbred calves, whereas the best conformation had Belgian Blue crossbred calves. It is recommended to crossbreed Brown breed dams, which offspring are not planned for replacement, with beef breed sires to improve carcass quality of slaughtered animals.*

(Keywords: crossbreeding, brown cattle, beef breeds, carcass traits)

### INTRODUCTION

In Slovenia dual purpose Brown breed cattle represents 10% of whole cattle population (Petrič *et al.*, 2009). In the last thirty years this dual purpose breed became more dairy-orientated. Milk yield in standard lactation increased in the last ten years for nearly 900 kg (Results of dairy ..., 2009). A possibility to improve carcass traits of Brown cattle animals designed for beef production is to cross dams with beef sires. In Slovenia, the most used beef breeds are Limousine (LIM), Belgian Blue (BB) and Charolais (CHA). The most of crossbred slaughtered animals in Slovenia are represented through crossbreeds between Brown breed and above mentioned beef breeds. The aim of our study was therefore to find out the effect of crossbreeding Brown dams with BB, CHA and LIM sires on growth and carcass performances of their offspring.

### MATERIALS AND METHODS

Data were collected from commercial slaughterhouses in Slovenia from January 2007 to December 2009. Data from slaughtered young bulls less than 24 months old (n=13.208), bulls older than 24 months (n=7.082) and calves up to 8 months of age and 185 kg carcass

weight (n=5.424) were taken into the study. Data of altogether 25,714 animals of the following genotypes were processed into statistical analysis: Brown breed (B) and crossbred animals Brown×Belgian blue (B×BB), Brown×Charolais (B×CHA) and Brown×Limousine (B×LIM). Net daily gain was calculated from hot carcass weight and age at slaughter. The conformation and fatness were estimated by independent controllers according to the EUROP classification system with subclasses, where the classes for conformation expressed with letters were transformed to the numbers (E+=15, E0=14,...,P=1) and classes for fatness as followed 1=1, 10=2, 1+=3,..., 5+=15. The data of each category were analysed by SAS, GLM procedure (2001). Genotype, year of slaughter and their interaction were included as fixed effects in the model. The differences among different genotypes were tested with CONTRAST statement.

## RESULTS AND DISCUSSION

The young bulls represent around 41% of all slaughtered animals in Slovenia (Žgur et al., 2009). The observed growth and carcass performances of young bulls are shown in *Table 1*. At slaughter the young bulls of different genotypes were of similar age. Brown bulls were on average 6.5 days older than the youngest genotype group. Carcass weight of all crossbreeds was significantly greater than that of Brown bulls. Among the crossbreeds the B×CHA bulls had the heaviest carcasses. The carcass weight of B×BB and B×LIM bulls was statistically not different. Similar results were found for net daily gain. In comparison to the Brown bulls, net daily gain of B×BB, B×CHA and B×LIM bulls was greater for 8.1%, 12.1% and 7.2%, respectively. Similar results were found in the study on growth and carcass quality of young bulls of Black and White breed crossed with beef breeds in Slovenia (Petric et al., 2009). Carcass conformation differed among all genotypes being the poorest for Brown bulls, which were on average classified in conformation class O+, and the best for B×BB bulls, which reached on average conformation class between R0 and R+. B×BB bulls were classified for almost 3 subclasses better than Brown bulls. Among the crossbreeds the B×LIM bulls had the poorest conformation, but still for almost two subclasses better than Brown bulls. Carcass fatness varied for almost one subclass between 5.8 (fatness class nearly 2+) and 6.7 (fatness class nearly 3-) among genotypes, being lowest for B×BB and highest for B×LIM bulls. Kögel et al. (1989a, b) reported on better carcass quality of crossbred bulls compared to purebred Brown cattle bulls. Results from positive effect of crossing another dual purpose breed (Simmental breed) with beef breeds are also known (Kögel et al., 2000a, b, 2001a, b).

Bulls older than 2 years (old bulls) represent 15% of all slaughtered animals in Slovenia (Žgur et al., 2009) which is 35% of all slaughtered bulls in the last three years. They were slaughtered at age 26.3 months on average (*Table 2*). Slaughter age of B×CHA group differed significantly downwards compared to Brown old bulls, while the slaughter age between other groups varied for a few days and was not significantly different. Carcass weight and net daily gain were significantly different between genotype groups. Brown old bulls had the lowest carcass weight and the lowest net daily gain, while the B×CHA old bulls were the heaviest and reached the best net daily gain. The difference between Brown and B×CHA old bulls was on average 38.3 kg for carcass weight and 54 g/day for net daily gain. Among crossbreds were B×LIM old bulls the lightest, which was the same in the case of young bulls. The conformation differed significantly among genotype groups of old bulls in the same order as in young bulls. The best conformation was valued for B×BB bulls with the conformation class R+,

following by B×CHA with half of subclass worse conformation and B×LIM with 0.7 subclass worse conformation. The poorest conformation was estimated for Brown old bulls (between O+ and R-), which was for more than 2.6 subclasses worse than in the B×CHA group. The lowest fatness had B×BB old bulls (on average the subclass between 20 and 2+); among other genotypes the difference was not statistically different and for 0.7 subclasses higher fatness as B×BB group (on average the fatness subclass between 2+ and 3-). Old bulls did not exhibited higher fatness than young bulls.

**Table 1**

**The number of bulls under 24 months of age in the genotype groups and their growth and carcass performances (Is mean $\pm$ SD)**

Genotype*	No. of observations	Traits				
		Slaughter age (days)	Carcass weight (kg)	Net daily gain (g/day)	Conformation**	Fatness**
B	8456	661.1 $\pm$ 0.8 <sup>a</sup>	330.6 $\pm$ 0.7 <sup>a</sup>	502 $\pm$ 1 <sup>a</sup>	6.30 $\pm$ 0.02 <sup>a</sup>	6.47 $\pm$ 0.02 <sup>a</sup>
B×BB	1653	656.8 $\pm$ 1.9 <sup>b</sup>	357.4 $\pm$ 1.5 <sup>b</sup>	547 $\pm$ 2 <sup>b</sup>	8.73 $\pm$ 0.04 <sup>b</sup>	5.78 $\pm$ 0.05 <sup>b</sup>
B×CHA	483	654.6 $\pm$ 3.5 <sup>ab</sup>	372.3 $\pm$ 2.7 <sup>c</sup>	572 $\pm$ 4 <sup>c</sup>	8.41 $\pm$ 0.08 <sup>c</sup>	6.34 $\pm$ 0.08 <sup>a</sup>
B×LIM	2616	657.3 $\pm$ 1.5 <sup>cb</sup>	354.6 $\pm$ 1.2 <sup>b</sup>	542 $\pm$ 2 <sup>b</sup>	8.15 $\pm$ 0.03 <sup>d</sup>	6.67 $\pm$ 0.03 <sup>c</sup>

\* B: Brown, BB: Belgian Blue, CHA: Charolais, LIM: Limousine; \*\* EUROP classification scoring: conformation: 15 (E+=best) to 1 (P=poorest); fatness: 1=1-, leanest to 15=5+, fattest; <sup>a,b</sup> values with different superscript among genotypes differ significantly ( $P<0.05$ ).

**Table 2**

**The number of bulls older than 24 months in the genotype groups and their growth and carcass performances (Is mean $\pm$ SD)**

Genotype*	No. of observations	Traits				
		Slaughter age (days)	Carcass weight (kg)	Net daily gain (g/day)	Conformation**	Fatness**
B	4799	804.9 $\pm$ 0.8 <sup>a</sup>	347.4 $\pm$ 0.8 <sup>a</sup>	433 $\pm$ 1 <sup>a</sup>	6.34 $\pm$ 0.02 <sup>a</sup>	6.44 $\pm$ 0.03 <sup>a</sup>
B×BB	762	801.9 $\pm$ 1.9 <sup>ab</sup>	376.0 $\pm$ 2.1 <sup>b</sup>	470 $\pm$ 3 <sup>b</sup>	8.95 $\pm$ 0.06 <sup>b</sup>	5.74 $\pm$ 0.07 <sup>b</sup>
B×CHA	209	794.8 $\pm$ 3.7 <sup>b</sup>	385.7 $\pm$ 4.0 <sup>c</sup>	487 $\pm$ 5 <sup>c</sup>	8.51 $\pm$ 0.12 <sup>c</sup>	6.34 $\pm$ 0.12 <sup>a</sup>
B×LIM	1312	804.4 $\pm$ 1.5 <sup>a</sup>	369.7 $\pm$ 1.6 <sup>d</sup>	461 $\pm$ 2 <sup>d</sup>	8.21 $\pm$ 0.05 <sup>d</sup>	6.42 $\pm$ 0.05 <sup>a</sup>

See Table 1

Animals slaughtered up to 8 months of age are included in the category calves, which represented 18.3% of all slaughtered bovine animals in Slovenia (Žgur *et al.*, 2009). 5424 calves slaughtered in the last three years were included into this study (*Table 3*). On average the slaughter age of all genotype groups was around 4 months, B×CHA calves were slaughtered the youngest but reached the highest net daily gain, and also statistically differed from all other genotype groups' net daily gain. The lowest net daily gain was estimated for Brown calves, 139 g/day less than B×CHA calves, which had also significantly lowest carcass weight. The carcass weight among other genotype

groups varied slightly and was on average around 10 kg higher than that of Brown calves. The best conformation score reached B×BB calves (subclass R0), calves from the groups B×CHA and B×LIM had for half subclasses worse conformation and the Brown calves for 2.3 subclasses worse conformation than B×BB calves. Fatness in all genotype groups was on average estimated around class 2-, compared to other groups the calves from B×LIM group had significantly higher fatness. *Dal Zotto et al.* (2009) reported that crossbreeding with beef bulls increased body weight, price and market value of calves from dairy and dual purpose dams, whereas the crossbreeding with BB bulls increased price and market value of calves much more than LIM.

**Table 3**

**The number of calves in the genotype groups and their growth and carcass performances (ls mean $\pm$ SD)**

Genotype*	No. of observations	Traits				
		Slaughter age (days)	Carcass weight (kg)	Net daily gain (g/day)	Conformation**	Fatness**
B	2934	125.8 $\pm$ 0.7 <sup>a</sup>	86.8 $\pm$ 0.4 <sup>a</sup>	731 $\pm$ 4 <sup>a</sup>	5.86 $\pm$ 0.03 <sup>a</sup>	4.23 $\pm$ 0.03 <sup>a</sup>
B×BB	742	126.7 $\pm$ 1.5 <sup>ab</sup>	97.8 $\pm$ 0.7 <sup>b</sup>	813 $\pm$ 7 <sup>b</sup>	8.23 $\pm$ 0.06 <sup>b</sup>	4.30 $\pm$ 0.05 <sup>a</sup>
B×CHA	231	116.3 $\pm$ 2.6 <sup>c</sup>	96.2 $\pm$ 1.3 <sup>bc</sup>	870 $\pm$ 13 <sup>c</sup>	7.61 $\pm$ 0.11 <sup>c</sup>	4.28 $\pm$ 0.09 <sup>a</sup>
B×LIM	1517	123.9 $\pm$ 1.0 <sup>b</sup>	95.1 $\pm$ 0.5 <sup>c</sup>	810 $\pm$ 5 <sup>b</sup>	7.80 $\pm$ 0.04 <sup>c</sup>	4.51 $\pm$ 0.04 <sup>b</sup>

See Table 1

## CONCLUSIONS

In conclusion, crossbred young and old bulls obtained better carcass weight and net daily gain than purebred Brown bulls. Among crossbred bulls the best growth performance showed B×CHA bulls in both age groups. Further, crossbred bulls reached on average for two subclasses better conformation. The best conformation was estimated for B×BB bulls, which had also the lowest fatness. Also crossbred calves slaughtered at around 4 months of age showed better carcass weight and net daily gain. All crossbred genotypes in all age groups showed better growth and carcass quality. From the results we can conclude that crossbreeding with CH improves the most growth traits while the crossbreeding with BB carcass traits.

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## The effect of wrapping veal carcasses in viscose foil on some carcass and meat quality traits

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### ABSTRACT

*Fifty veal calves were used to study the effect of wrapping carcasses in viscose foil on carcass and meat quality traits. Half of them were wrapped in viscose foil before they were exposed to chilling, and half of them served as a control. Veal calves were around 4 months old at slaughter and reached on average 84 kg warm carcass weight. After 24 hours of conventional chilling wrapped veal calves had 0.45 kg or 0.5% lower ( $P<0.001$ ) chilling loss and 0.1 lower ( $P<0.001$ ) pH value in longissimus dorsi muscle. Wrapping had no significant ( $P>0.05$ ) effect on meat colour of flenk muscle.*

(Keywords: calves, chilling, meat quality)

### INTRODUCTION

Veal meat production is an important part of cattle production in Slovenia. In 2009 from 36,943 t of total beef and veal meat harvested in abattoirs, 5.9% represented veal meat (SURS, 2010). Slaughtered calves represented 19.4% of total number of slaughtered cattle. Veal meat production in Slovenia is characterised by slaughtering of very young calves. The average carcass weight of slaughtered calves in Slovenia in 2009 was only 89 kg. The main reason for slaughtering so young and light calves is that consumers favour light coloured pink meat.

Valin (1991) stated that veal colour was always considered as a quality characteristic. Moreover, tenderness was the most important veal quality characteristic at the consumer level. Also Glitsch (2000) found out in the consumer survey, carried out in six European countries, that meat colour is one of the most important intrinsic quality indicators of meat. Meat colour is defined by many factors like animal genetics, ante- and post-mortem conditions, fundamental muscle chemistry, and many factors related to meat processing, packaging, distribution, storage, display, and final preparation for consumption (Mancini and Hunt, 2005).

There are numerous publications concerning veal feeding and well being due to the fact that in the past a number of changes in veal meat production system occurred (Ngapo and Gariepy, 2006). The most important change was the introduction and acceptance of grain-fed, heavier calves and a move from individual pens to group housing owing to well being of animals and public perception of such production systems. Proper consumer education towards the purchase of welfare friendly beef and veal meat as stated by Cozzi *et al.* (2009) appears to be one of the most important tools for further development of animal friendly production systems. Consumers give increasing importance to the extrinsic quality attributes of meat in response to increasing concerns on safety, health, ethical factors, etc. (Bernues *et al.*, 2003), which seems promising.

On the other hand less attention has been devoted to post-slaughter management of veal calves carcasses. Chilling rate affects carcass weight losses as well as meat quality traits together with the rate and extent of pH-fall (Honikel, 2004a, Savell *et al.*, 2005). Adjusted pH-fall and temperature-fall are extremely important to prevent cold shortening (Honikel, 2004b). This is of special importance in calves because they chill very rapidly due to their low carcass weight and absence of significant subcutaneous fat.

In our paper we study the effect of wrapping veal carcasses in viscose folia to prevent rapid chilling on carcass chilling losses and meat colour.

## **MATERIALS AND METHODS**

Fifty veal male Holstein calves were slaughtered in commercial slaughterhouse in a common procedure. Thirty minutes after slaughter warm carcass weight was recorded and veal calves were randomly arranged into two groups. Half of the veal calves were wrapped in viscose foil. Wrapping was started at hind shank and continued around the carcass and downwards to the neck to completely cover the carcass and to make some kind of carcass isolation. The other half of the veal calves served as a control. Conformation and fatness were estimated according to EUROP system. Carcasses of the veal calves were subsequently moved into chilling room. They were conventionally chilled for the next 24 hours (temperature 0 °C, wind speed 0.5 m/s). After 24 hours, carcasses were weighted again and chilling losses were calculated. pH<sub>24</sub> (Metler, Toledo) was measured in *longissimus dorsi* muscle between last thoracic and first lumbar vertebra. Meat colour was measured on flenk muscle with Minolta CR300 colorimeter and expressed as CIE Lab values. Statistical analysis was performed by SAS statistical package (SAS, 2001) with TTEST procedure.

## **RESULTS AND DISCUSSION**

In *Table 1* carcass traits of veal calves are presented. Veal calves were about 4 months old at slaughter and had 85 kg warm carcass weight in wrapped and 82 kg in unwrapped group. There were no significant differences between both groups of veal calves. Estimated conformation was 2.4 on average (between O and R class) in both groups. All slaughtered veal calves were classified into fatness class 2. Chilling loss in unwrapped group was 1.78 kg or 2.09%, whereas in wrapped group amounted only to 1.33 kg or 1.60%. Both differences were highly (P<0.001) significant. These chilling losses are relatively low compared to those reported by Mandell *et al.* (2001). They reported chilling losses from 2.4 to 3.1% in veal carcasses from 164 to 201 kg hot carcass weight. Pommier *et al.* (1995) reported chilling losses from 1 to 1.4%, but for veal calves with hide-on and hot carcass weight around 124 kg. So chilling losses found in present experiment are intermediate between those reported by Pommier *et al.* (1995) and Mandell *et al.* (2001).

In *Table 2* mean value for pH<sub>24</sub> value and meat colour are presented. pH<sub>24</sub> value in *longissimus dorsi* muscle was in unwrapped group 5.95 and in wrapped one 5.85. The difference between both groups was highly (P<0.001) significant. Hence the wrapping of the carcasses influenced the rate of pH-fall. In contrast, wrapping had no effect on meat colour measured on abdominal muscle. There were no significant differences between both groups in L, a and b values. Similar results were found by Klont *et al.* (1999) who reported that veal carcass colour was not related to the observed variation in post-mortem pH and temperature in the *longissimus lumborum* muscle.

**Table 1**

**Mean value and standard deviation for some carcass and meat traits of wrapped and unwrapped veal calves**

	Unwrapped n=25		Wrapped n=25		Effect of wrapping
	$\bar{x}$	SD	$\bar{x}$	SD	
Age, days	124	16	130	21	NS
Warm carcass weight, kg	85.28	6.50	82.52	6.62	NS
EUROP - conformation <sup>1</sup>	2.40	0.50	2.40	0.50	NS
EUROP – fatness	2.00	0	2.00	0	NS
Cold carcass weight, kg	83.50	6.47	81.19	6.47	NS
Chilling losses, kg	1.78	0.32	1.33	0.34	***
Chilling losses, %	2.09	0.39	1.60	0.37	***

<sup>1</sup>E=5, U=4, R=3, O=2, P=1; NS: P>0.05; \*\*\* P<0.001

**Table 2**

**Mean value and standard deviation for pH<sub>24</sub> and meat colour of wrapped and unwrapped veal calves**

	Unwrapped n=25		Wrapped n=25		Effect of wrapping
	$\bar{x}$	SD	$\bar{x}$	SD	
pH <sub>24</sub>	5.95	0.08	5.85	0.09	***
Meat colour, CIE L	50.10	3.46	50.71	3.76	NS
a	12.47	2.28	12.40	2.91	NS
b	6.07	2.11	5.65	3.15	NS

NS: P>0.05, \*\*\* P<0.001

## CONCLUSIONS

On the basis of the presented results we can conclude that wrapping of veal carcasses in viscose folia can diminish chilling losses for about half percent. At the same time it accelerated post mortem glycolysis in *longissimus dorsi* muscle but it had no effect on meat colour of flank muscle.

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## Trentingrana cheese production: analysis of dairy systems

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### **ABSTRACT**

*This research aimed to study the relationships between livestock systems and typical products in the Trento Autonomous Province, a mountainous area of the Eastern Italian Alps. A total of 1111 dairy farms were censused in the province; a sample of 678 was surveyed collecting information on herd composition in terms of animal breeding and category, management system and quality of milk. Data were analyzed with a non-hierarchical cluster procedure that clustered farms into 6 types: modern farms with traditional feeding (86 farms); small farms with corn silage (51 farms); modern farms with unifeed and corn silage (63 farms); traditional small farms without summer pasture (146 farms); intensive farms (34 farms); traditional small farms (298 farms). The traditional systems are able to maintain a greater animal biodiversity than the intensive ones, thanks to the farming of indigenous breeds. This group of farms is largely involved in the Trentingrana cheese production, a traditional long ripened cheese (DOP) of the area, whereas the intensive systems produce milk for large distribution. This study demonstrates that in the province there is a wide variability of livestock farming systems. Traditional systems which have an important role for maintenance of landscape and farmed biodiversity are strongly connected with the typical cheese production, and this association could help in maintaining their economic viability.*

(Keywords: farming system, cluster analysis, mountain areas, dairy cattle, Trentingrana cheese)

### **INTRODUCTION**

In mountainous areas, physical disadvantage and extreme distances reduce competitiveness of livestock systems, and give rise to serious limits to the technical and structural adjustment. In addition, people of mountain are less adaptable because of entrenched traditions (Walther, 1986; Campagne *et al.*, 1990; Bazin, 1995). In the alpine regions of southern Europe traditional farming systems, with low production and incomes, have therefore experienced a dramatic decline (MacDonald *et al.*, 2000). The case of the province of Trento, in the north-eastern Italian Alps, is an example of this trend. Livestock farming was in the past a fundamental source of income for the population, which had permeated social and cultural traditions and had shaped a typical cultural landscape (MacDonald *et al.*, 2000; Sturaro *et al.*, 2005).

However, during the last 40 years the number of farms and livestock has dropped dramatically (Walther, 1986; MacDonald *et al.*, 2000). In the attempt of maintaining economic viability, the remaining farms have pursued an intensification process, with an increase in the average number of animals farmed/unit and a substitution of indigenous breeds with more productive breeds, such as Holstein Frisian cows (Battaglini *et al.*, 2003). Pasture and meadows which were abandoned with the closure of traditional farms

were not reutilized by the new intensive farms, with the result of an extensive spontaneous reforestation and a loss of landscape attractiveness (Gusmeroli and Della Marianna, 2005; Cocca et al., 2007).

In fact, maintenance of traditional farming systems based on grasslands, such as permanent meadows and alpine summer pastures, is seen today as an essential strategy for safeguarding mountain landscape and biodiversity (Mac Donald et al., 2000; Battaglini et al., 2003; Sturaro et al., 2005; Cocca et al., 2007). In addition to local policies for agricultural subsidies supporting extensive farming, also the economic valorization of typical dairy products may help to sustain traditional farming systems. In the Trento province there are several types of cheeses, and Trentingrana represents the most important of them. This product is part of the Grana Padano family (Aprea et al., 2007), but the trademark is different, and it is a DOP product (a recognised quality trademark). Even if the production of Trentingrana is relatively small (about 4000 ton/year; consortia personal communication) when compared with that of the direct competitors (about 125,000 ton/year for Grana Padano; and about 113,000 ton/year for Parmigiano; <http://www.granapadano.com>, [www.crpait](http://www.crpait)), it is relevant for the local economy. Trentingrana is produced with partly skimmed, raw cows' milk acidified with a mixture of *Lactobacillus* and coagulated by the addition of bovine liquid rennet but, having a more restrictive production protocol, the use of lysozyme is not allowed. The ripening process is divided in two phases: the first lasts nine months and takes place in the dairies, the total period lasts 15 to 20 months and the second phase is done in a Trentingrana storehouse. At the end of both periods the product is thoroughly checked. The rounds whole cheese not fit for sale are discarded.

This paper is part of a larger research project, aimed to monitor the production chain of Grana Trentino. The specific aim of this paper is to examine the management of dairy farms in the province of Trento conferring the milk for Trentingrana cheese production.

## MATERIALS AND METHODS

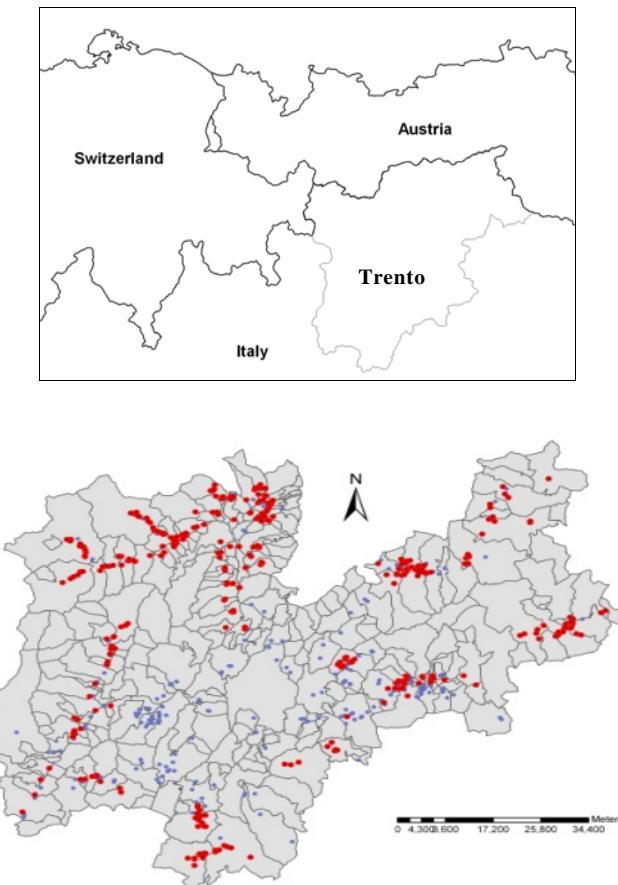
The survey was carried out in Trento that is an autonomous province of northern Italy (*Figure 1*). This province consists of 223 municipalities, all classified as mountainous for the national statistical database (ISTAT, 2007), covering an area of 6200 km<sup>2</sup> with a minimum altitude of 66 m asl and maximum of 3769 m asl. Vegetation is predominantly characterized by woodland (66%), followed by meadows and pastures (26%). Permanent and arable crops represent respectively 5% and 1% (ISTAT, 2002 and 2007). Farms with livestock account for 14% of the total holdings of the province, but with a heterogeneous distribution across the territory (*Figure 1*). In the areas interested by Trentingrana cheese production, livestock farming accounts for 40–50% of farms.

To study the characteristics of dairy systems in Trento, different databases were merged to obtain the final database. A first database, provided by the Provincial Agency for payments (APPAG), contained data on type of farm, with 1111 dairy cow herds out of a total of 2153 farms, and herd composition with information on livestock category and breeds (Brown Swiss, Holstein Friesian, Simmental, Rendena, Alpine Grey or crossbreeds). These data were merged with those of the cattle population register with a number of 1534 farms (both dairy and beef) provided by the veterinarian service of the Province (APSS), which included the farms location (georeferenced on a GIS). Then, main structural characteristics and feeding techniques of farms (see below for details) were collected by personnel of the Federazione Provinciale Allevatori Trento (FPAT) on

719 farms, and included in the database. Finally, the database was completed with the milk recording data (886 holdings; of which 724 with more than 100 controls) produced by the Federation itself using the test-day model. From the final data editing 678 farms with complete information were retained for statistical analysis. Qualitative aspects considered for the milk given to dairies are: fat, protein (and casein), lactose percentage and somatic cells score. These characters refer to bulk milk and are measured with the controls from the CONCAST-Trentingrana. In *Figure 1* the distribution of sampled farms on the territory are reported.

**Figure 1**

**The Autonomous Province of Trento and location of farms in the territory**



Red points indicate dairy farms conferring to Trentingrana, blue points other dairies

Farms were grouped by structural and management characteristics by adapting the “Non Hierarchical K-means clustering” (SAS, 2006). Observations were allocated to the groups based on the smallest Euclidean distance from the initial seeds of the cluster. Cluster centroids were updated as each observation was assigned (Ottaviani *et al.*, 2003; Usai *et al.*, 2006). The method maximizing homogeneity inside a group and diversity

within the groups offered different protocols to decide how many groups would have parted from the initial distribution. The variables included in the analysis are: housing (tie vs free stalls), feeding system (Unifeed vs traditional), use of Summer Pasture (yes/no), Corn Silage (yes/no) and Livestock Unit (LU)/Farm. The profiles of each cluster were used to investigate the differences between clusters. Proportion of each breed in the herd, average milk production and milk quality were compared among groups by using a one-way ANOVA (SAS, 2006). To verify whether there was association between farming systems and Trentingrana cheese production, the distribution across identified systems of farms conferring to Trentingrana was compared to that of the total farms sample.

## RESULTS AND DISCUSSION

Six different farming styles were identified from the non hierarchical cluster analysis of the 678 sampled farms (*Table 1*). The number of clusters was chosen on the base of cubic clustering criteria ( $F=431.57$ ,  $R^2=0.67$  and cubic clustering criteria=30.55, data not in table). The identified clusters were characterized by very specific features. The first is characterized by modern farms ( $n=86$ ) with large herds (80 LU/Farm), mainly free housing and traditional feeding technique with no use of corn silage and a frequent use of summer pastures. The second group identifies 51 farms with an average herd size (42 LU/Farm), traditional tie stall housing (96%), use of both traditional and unifeed (41%) feeding technique but in all cases of corn silage (100%), and a low frequency of use of summer pastures (14%). The third cluster grouped 63 farms with a large herd size (99 LU/unit), modern housing (tie stall only 13%) and feeding technique (unifeed 100%), almost no use of summer pasture (10%), and an average frequency (49%) of corn silage use. The fourth group (146 holdings) is characterized by a small herd size (32 LU/farm), traditional housing (90% tie stalling) and feeding practice with no unifeed (3%) and corn silage (0%); summer pastures are also absent (0%). The fifth cluster groups 34 intensive farms similar to those of the most productive lowlands of Italy, with a very large herd size (241 LU/farm), modern housing (100% free) and feeding (91% unifeed) and frequent use for corn silage (65%). The sixth cluster is very similar to the fourth one, differing only for the use of summer pastures (100%).

**Table 1****Profiles of groups identified by the non hierarchical cluster analysis**

Cluster	n	Average LU/Farm (SD)	Tie Stalling %	Unifeed %	Summer Pasture %	Corn Silage %
1	86	79.8 (29.5)	22	10	70	1
2	51	42.1 (25.9)	96	41	14	100
3	63	99.4 (31.2)	13	100	10	49
4	146	31.9 (20.5)	90	3	0	0
5	34	241.1 (81.7)	0	91	21	65
6	298	21.6 (16.9)	98	0	100	0

Clusters: 1: Modern farms with traditional feeding; 2: Small farms with corn silage; 3: Modern farms with unifeed and corn silage; 4: Traditional small farms without summer pasture; 5: Intensive farms; 6: Traditional small farms

The differences between the clusters for proportion of different breeds, productivity and milk quality were significant for all variables (*Table 2*). In the Trento province is quite common to have two or more breeds in the same farm, with Brown Swiss and Holstein Friesian accounting for a main proportion in all clusters (the two breeds together accounted for 54 to 93% of LU's according to the cluster), with Holstein Friesian predominating over Brown Swiss in the more intensive systems. Simmental, Rendena and Alpine Grey were almost absent in these systems (clusters 3 and 5: 2 and 11%), while they were present with appreciable proportions in the other, traditional systems (from less than 20% in clusters 1 and 2 to 40% in cluster 6).

**Table 2**

**Analysis of differences between farming systems  
in terms of reared breeds, milk production and quality**

	<b>1 (SD)</b>	<b>2(SD)</b>	<b>3 (SD)</b>	<b>4 (SD)</b>	<b>5 (SD)</b>	<b>6 (SD)</b>	<b>F</b>	<b>P</b>
Brown Swiss (%)	54 (35)	36 (32)	35 (29)	47 (34)	24 (31)	46 (40)	5.17	< 0.001
Holstein Frisian (%)	20 (26)	42 (35)	58 (31)	20 (26)	61 (38)	7 (19)	67.64	< 0.001
Simmental (%)	11 (21)	7 (13)	2 (6)	15 (25)	5 (15)	15 (25)	4.83	< 0.001
Rendena (%)	8 (26)	7 (21)	-	3 (15)	6 (23)	12 (29)	4.47	< 0.001
Alpine Grey (%)	-	3 (13)	-	7 (23)	-	13 (29)	7.54	< 0.001
Milk (l)	23.3 (3.6)	18.9 (4.2)	25.8 (4.3)	19.3 (4.4)	27.7 (4.1)	17.7 (3.9)	81.84	< 0.001
Fat (%)	3.92 (0.24)	4.00 (0.23)	4.00 (0.24)	3.95 (0.24)	4.00 (0.32)	3.90 (0.23)	3.57	< 0.01
Casein (%)	2.75 (0.14)	2.68 (0.14)	2.75 (0.13)	2.73 (0.14)	2.76 (0.12)	2.67 (0.14)	8.71	< 0.001
Somatic Cell Score	3.22 (0.63)	3.62 (0.76)	3.24 (0.63)	3.12 (0.86)	3.26 (0.59)	3.21 (0.92)	2.82	< 0.05

Milk production ranges from a minimum of 18 kg/d in traditional farms to a maximum of 26 kg/d in intensive ones (*Table 2*). Fat and casein content and somatic cell score varied slightly between types of farm, with a general high quality, most probably due to the guidelines for Trentingrana cheese production.

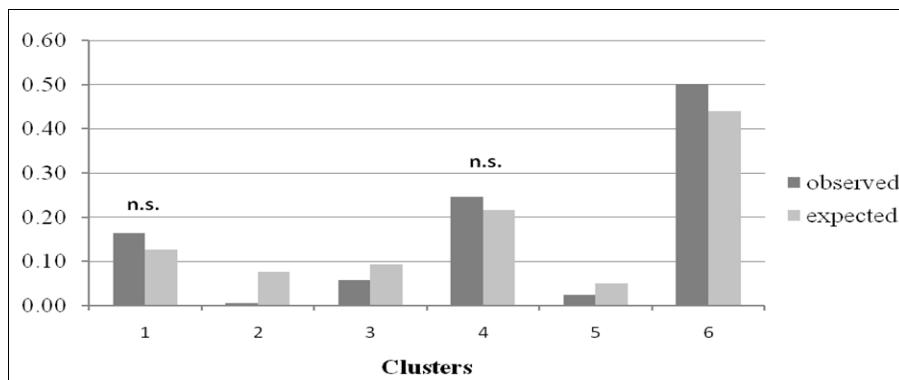
The above results confirm a general dichotomy between traditional farming systems, with small units based on use of grassland, and intensive systems with large farms based on modern feeding techniques and corn silage. Other studies conducted in the province at a farm scale (*Marini et al.*, 2009), in Austria (*Schmitzberger et al.*, 2005) and in the province of Belluno, bordering that of Trento (*Giupponi et al.*, 2006) at a landscape scale, indicate that in mountain areas highly-producing farms support the lowest biodiversity and landscape richness. In addition, it is clear that biodiversity of farmed breeds is also much greater in traditional systems.

*Figure 2* shows that the distribution in the six clusters of the 414 farms delivering milk to Trentingrana differed significantly from that of the total sample of 678 sampled

farms ( $\chi^2=48.43$ ;  $df=5$ ;  $P<0.001$ ). As respect to what expected from the total sample of 678 farms, traditional farming systems based on grasslands were more frequent and modern, intensive systems with less use of grasslands and more use of corn silage (which is forbidden in the Trentingrana guidelines) were less frequent. This means that the role of small traditional farms is not fundamental for conservation of the landscape and biodiversity, but also for supporting typical products such as Trentingrana.

**Figure 2**

**Distribution in the 6 farming systems of farms delivering to Trentingrana (observed) as respect to that expected from the distribution of the total farms (expected)**



## CONCLUSIONS

The dairy sector of the Trento province is diversified into 6 different farming systems which differ in terms of structures, feeding techniques, farmed breeds and production level. Only milk quality is homogenous, and good, amongst systems. Traditional farms clusters, with feeding techniques based on grazing and on-farm produced forage, maintain local breeds with low productivity and are largely involved in the Trentingrana cheese production. On the opposite, modern and intensive farms which rely mostly on corn silage and maintain highly productive breeds are excluded from the production of this cheese. The association between extensive dairy farming and typical cheese production might increase the economic viability of traditional systems, and as a consequence help in maintaining a sustainable livestock sector in mountain areas. A prospective of this research will be the analysis of the relationship between dairy farms, environmental impact and landscape maintenance in Trento province.

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## Factors affecting blood parameters of autochthonous Cika cattle

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### ABSTRACT

*Cika cattle is the only Slovenian autochthonous cattle breed, which has been preserved to the present days. The aim of this study was to determine the blood parameters of Cika cattle regarding the variability in phenotypes and age of Cika cattle. This study included 122 Cika cattle of two phenotypes (71 Cika and 51 Pinzgauer phenotypes) from different farms and regions of Slovenia. There were 108 cows and 14 sires for natural mating. The average age of sires at sampling were 2.0 years, the average age of cows at sampling was 8.6 years. Farms with Cika cattle were placed from 200 m to 1100 m above the sea level with an average altitude of 643.5 m. The number of erythrocytes (RBC), leucocytes (WBC) and platelets (PLT) and values of haemoglobin (Hb), haematocrit (Ht), the mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC) were measured with haematological analyser ABC Vet. Data were analysed by GLM procedure of statistical package SAS/STAT considering phenotype and sex as fixed effects as well as animal age as linear regression. Analysis of variance proved that the sex significantly affected almost all included blood parameters of Cika cattle except MCHC. The effect of age at the day of blood collecting affected the RBC, WBC, values of Hb and Ht, while the effect of phenotype affected only the MCHC.*

(Keywords: cattle, Cika, autochthonous breed, blood parameters)

### INTRODUCTION

Cika cattle is the only Slovenian autochthonous cattle breed, which has been preserved to the present days. On June 1<sup>st</sup>, 2009 the Cika population accounted 2159 animals. Considering phenotype the population of Cika cattle is very heterogeneous. However, all animals are divided in three different phenotype groups for the selection purposes. Only two groups were included in this study the Cika and the Pinzgauer phenotype. Cika phenotype includes animals of smaller autochthonous phenotype, while Pinzgauer phenotype includes larger animals with a high share of Pinzgauer genotype as a result of artificial insemination with the semen of Pinzgauer sires after the Second World War.

Cika cattle are usually reared extensively in the cow-calf system for beef production mostly. They are to a smaller extent still used for milk production in traditional regions for Alpine dairy-farming. The largest share of Cika population is reared very extensively on small farms without the addition of any concentrates. In most cases farms with Cika cattle are placed on very high altitude above the sea level. The animals are grazing in the summer and are fed by hay in winter time. About one third of the farms with Cika cattle have the organic farming production. Cika cattle are perfectly adapted to grazing in difficult

mountain environment, such as rough climatic conditions, meagre food resources and long distances to get to the summer pastures over 1000 m above the sea level. They have an excellent ability to exploit voluminous forage and the persistency to graze on steep mountain pastures. A lot of cows have high longevity (Simčič, 2008).

In veterinary medicine, haematological examinations present an effective tool in monitoring the health and nutritional status of animals. We investigated the blood parameters in autochthonous Cika cattle because of a very extensive rearing system compared to the intensive rearing high productive cattle breeds. The aim of this study was to establish the blood parameters of Cika cattle regarding to the variability in phenotypes, as well as ages and sex.

## MATERIALS AND METHODS

This study included 122 Cika cattle. There were 108 cows and 14 sires for natural mating of two phenotypes; 71 Cika and 51 Pinzgauer phenotypes. The distribution of cattle by sex was not in equilibrium due to a low number of larger herds which use sires for natural mating. Most of the breeders had a low number of cows in the herd and they preferred artificial insemination.

There was also a great variability in cattle age. The average age of cows was 8.6 years at sampling. The youngest cow had 3.2 years, while the oldest 18.0 years. The average age of sires at sampling was 2.0 years, where the youngest sire had 1.0 year and the oldest 3.0 years. Sires used in herds and included in sampling were much younger than cows, because the breeders use a sire just for one or two years in a herd mainly to prevent inbreeding in small population of autochthonous Cika cattle. The Cika cattle breed are known for its longevity and this is the reason for a great variability in cow's ages.

Cika cattle were housed in different farms and regions throughout Slovenia with altitude ranging from 200 to 1100 m above the sea level. Cika breed is known as a low production breed like other autochthonous cattle breeds. This breed is kept very extensively, the base forage being grazing in the summer and hay in the winter time, without supplemented concentrates. The exact production level in the farms was not estimated because of too much missing data (they are mostly used for cow-calf system).

Blood samples were taken in the spring in year 2008 from the tail vein in evacuated tubes with EDTA. The number of erythrocytes (RBC), leucocytes (WBC) and platelets (PLT), and the values of haemoglobin (Hb), haematocrit (Ht), the mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC) were measured with haematological analyser ABC Vet (Horiba ABX, Montpellier, France).

Data were analysed by GLM procedure of statistical package SAS/STAT (SAS, 2001) considering phenotype and sex as fixed effects and animal age as linear regression (model 1). Pre-analysis had shown that the effect of farm altitude did not significantly affect the investigated blood parameters.

$$y_{ijk} = \mu + T_i + S_j + b_l(x_{ijk} - \bar{x}) + e_{ijk} \quad (1)$$

Where:

$y_{ijk}$  = blood parameters (RBC, WBC, PLT, Hb, Ht, MCH, MCHC);

$T_i$  = phenotype;  $i$  = Cika, Pinzgauer;

$S_j$  = sex;  $j$  = sire, cow;

$x_{ijk}$  = age at blood samples collecting, months;

$e_{ijk}$  = residual.

## RESULTS AND DISCUSSION

The average values of haematological variables in Cika cattle included in this study (*Table 1*) were within reference intervals used for cattle. The differences in blood parameters between Cika and Pinzgauer phenotype of Cika cattle were not significant except for the mean corpuscular haemoglobin concentration (MCHC) (*Table 2*). The animals of smaller Cika phenotype had in average 5.36 g/L higher MCHC compared to the larger animals of Pinzgauer phenotype. *Mayrhofer et al.* (1976) studied haematological variables of 750 Pinzgauer cattle in Austria and established very similar mean values as in Pinzgauer phenotype of Cika in this study (*Table 1*). They ascertained mean number of RBC  $6.54 \times 10^{12}/L$ , WBC  $7.03 \times 10^9/L$ , mean value of Hb 114 g/L and Ht 0.34 L/L. Some animals of Pinzgauer phenotype of Cika cattle still have large part of Pinzgauer genotype as a result of artificial inseminations of Cika cows with the semen of Pinzgauer sires from Austria after the Second World War.

**Table 1**

**Blood parameters of Cika cattle by phenotypes and sex**

Blood parameter	Phenotype (LSM±SE)		Sex (LSM±SE)	
	Cika (n=71)	Pinzgauer (n=51)	Sires (n=14)	Cows (n=108)
RBC $\times 10^{12}/L$	$6.42 \pm 0.13$	$6.33 \pm 0.18$	$6.95 \pm 0.26$	$5.81 \pm 0.08$
WBC $\times 10^9/L$	$8.14 \pm 0.29$	$7.51 \pm 0.39$	$8.46 \pm 0.58$	$7.20 \pm 0.18$
PLT $\times 10^9/L$	$266.32 \pm 24.82$	$264.07 \pm 33.85$	$209.61 \pm 49.85$	$320.78 \pm 15.26$
Hb (g/L)	$118.39 \pm 2.36$	$115.11 \pm 3.22$	$122.35 \pm 4.73$	$111.15 \pm 1.45$
Ht (L/L)	$0.34 \pm 0.07$	$0.34 \pm 0.01$	$0.36 \pm 0.01$	$0.32 \pm 0.05$
MCH (pg)	$18.65 \pm 0.20$	$18.56 \pm 0.27$	$18.17 \pm 0.39$	$19.04 \pm 0.12$
MCHC (g/L)	$350.13 \pm 1.49$	$344.77 \pm 2.03$	$344.60 \pm 3.00$	$350.30 \pm 0.92$

n: number of samples, LSM: least square means, SE: standard error

Sex of animals significantly affected all investigated blood variables except MCHC value (*Table 2*). Similar results were established also by *Klinkon et al.* (2009). In the research of *Mayrhofer et al.* (1976) the sex influenced significantly only the value of Ht. In sires in this study higher mean values of RBC, WBC, Hb and Ht and lower mean values of MCH, MCHC and PLT were measured compared to cows. Similarly, *Stark et al.* (1978) also found higher values of Hb in Friesian bulls compared to Friesian cow blood samples from the United Kingdom. *Klinkon* (1992) established higher values of Hb and RBC and lower values of MCH in bulls in comparison to cows in Slovenia. *Straub* (1981) reported that bulls have  $1.0$  to  $1.5 \times 10^{12}/L$  greater number of RBC than cows. However, in this study the lower number of included sire blood samples should be considered. The whole population (2159 animals) of autochthonous Cika cattle is reared by 412 breeders with an average number of 2.2 cows per herd. The number of herds with more than five Cika cows was 26 and just in some of them a sire was reared for natural mating. All the above mentioned factors are the reason for such low number of included sires in the blood sampling.

The age of animals had statistically significant influence on the number of RBC and WBC, as well as on Hb and Ht values. The values of variables were decreased by animal age. The number of RBC decreased in average for  $0.04 \times 10^{12}/L$  per year, WBC for  $0.19 \times 10^9/L$  per year, the value of Ht for 0.23 L/L per year and Hb for 0.08 g/L per

year. *Klinkon et al.* (2009) found that in Cika cows the number of RBC and WBC as the values of Hb and Ht decreased with age while the number of PLT and the values of MCH and MCHC increased. Likewise, *Stark et al.* (1978) determined decreased values of RBC by age in Friesian bulls. Influence of age on the values of haematological parameters of cattle species was ascertained also in other studies (*Feldman et al.*, 2006; *Mammerickx et al.*, 1978; *Klinkon*, 1992; *Mayrhofer et al.*, 1976). The general trend is that RBC, Hb and PCV values are high at birth and decline with age over the first 6 months to 1 or 2 years of life, although considerable differences are found in values reported by various investigators. By 2 to 4 years of age various red cell parameters tend to increase slightly and then stabilize. However, RBC counts may continue to decline for 5 to 6 years of age before becoming stabilized, while Hb and PCV may remain constant irrespective of age (*Straub*, 1981; *Klinkon*, 1992). On the other hand, *Ciaramella et al.* (2005) determined haematological profile of 100 Mediterranean buffalos ranging in age from two to 14 years. They found decreased values of RBC, WBC and Hb by ages similar to Cika cattle in this study. However, all haematological values obtained were comparable with the normal values in adult cattle.

**Table 2****Analysis of variance for blood parameters of Cika cattle by GLM**

Blood parameter	n	p-values			R <sup>2</sup>
		Phenotype	Sex	Age	
RBC	122	ns	<0.0001	0.0303	0.27
WBC	122	ns	0.0436	<0.001	0.29
PLT	122	ns	0.0384	ns	0.04
Hb	122	ns	0.0284	0.0368	0.15
Ht	122	ns	0.0161	0.0478	0.15
MCH	122	ns	0.0401	ns	0.11
MCHC	122	0.0038	ns	ns	0.11

n: number of samples, R<sup>2</sup>: coefficient of determination

Proportion of variability explained with the model 1 (*Table 2*), was the highest and similar for the WBC and RBC. Very similar coefficients of determination (R<sup>2</sup>) were at values of Hb, Ht, MCH and MCHC. The lowest proportion of variability explained had PLT. The effects included in the model 1 were the most accurate and known. On the other hand, there were a lot of effects which are very difficult to determine and estimate. One of them was the effect of the breeder and consequently rearing and feeding technology on the farm. Also the production level and reproduction status of animals were not known. The large part of non estimated effects could explain lower coefficients of determination.

Although the breed has an effect on the values of haematological variables (*Feldman et al.*, 2006; *Klinkon*, 1992; *Mayrhofer et al.*, 1976), there were the values of haematological variables established in Cika cattle within reference intervals used for the interpretation of result in clinical pathology (*Jazbec*, 1990; *Feldman et al.*, 2006).

**CONCLUSIONS**

Irrespective of the fact that Cika cattle is an autochthonous and low productive breed the established values of haematological variables were within reference intervals used for

cattle which were prepared on the basis of cattle from larger population of widely used cattle breeds. Despite the well known differences in body shape between Cika and Pinzgauer phenotype there were no significant differences in blood parameters with the exception of the MCHC value. Sex of animals influenced significantly all investigated blood variables except MCHC value. The age of animals had a significant influence on the number of RBC and WBC as also on values of Hb and Ht. The values of mentioned variables were decreased by the animal age.

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## Blood profile in cows from small farms with low productivity

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### ABSTRACT

*The aim of the study was to analyse the results of haematological and biochemical examinations of blood in cows from farms with low rearing intensity. The results of blood examinations from 51 cows were analysed. The cows originated from different small farms from south east part of Slovenia. In blood samples haematological and different biochemical variables were measured. The results were evaluated regarding the normal values for cattle. The descriptive statistics and percentage of cows deviating from normal values for investigated variables were calculated. The mean values of investigated variables were inside reference interval for cattle except the portion of eosinophils which was above the reference value. In 16.7% of cows haemoglobin concentration and in 31.0% haematocrit value was below the reference value. In 55.6% of cows eosinophilia was ascertained. The results of biochemical examination show that 45.2% of cows had a concentration of total serum protein, 28.8% a concentration of urea and 35.5% a concentration of serum iron below the reference value. Some deviations were observed also regarding the mineral status. The results indicate inappropriate diet of these cows and in many cases possible infestation with parasites. Better care in parasite control and a well balanced diet would increase production in such herds.*

(Keywords: cows, blood, haematology, biochemistry, low intensity of production)

### INTRODUCTION

Extensive rearing of cattle is used predominantly in suckling cows systems and for beef production. Different studies investigated growth of extensively reared cattle (*Marino et al.*, 2009; *Pavlik et al.*, 2009; *Lewis et al.*, 1990). Some studies researched also the pasture based milk production systems (*Cavestany et al.*, 2005). Small farms usually produce milk and rear calves. Their productivity is quite low and the diet is forage based with low quantities of concentrates. In regions where the intensive farming is not possible they are important for the preservation of cultivated landscape.

The blood examinations like haematological and clinical chemistry profile are a valuable diagnostic tool for the evaluation of nutritional and health status of cattle. There are some blood metabolites which are related to the nutritional status of the cattle, they represent animal response to the nutrition. Blood variables related to protein status include total serum proteins and urea. Urea levels, in relation to nutrition vary according to protein content, protein degradability, non protein nitrogen and energy of the diet (*Park et al.*, 2002; *Russel et al.*, 2007). Phosphorus has no direct mechanism of regulation, although calcium regulating hormones directly affect its blood concentration. Calcium and phosphorus have important bone reserves, while the magnesium reserve is low and has no primary hormonal response for the compensation (*Martens and Schweigel*, 2000; *Larsen et al.*, 2001).

The present study intended to establish a mutual connection between the relevant blood variables and potentially valuable background information about the cows from small farms with low production intensity. For this purpose we analysed the results of haematological and biochemical analyses of blood in cows from small farms.

## MATERIALS AND METHODS

In the present study the results of haematological and biochemical examination of blood samples from 51 cows, predominantly of combined breeds (Brown Swiss, Simmental) which were sent to our clinical laboratory were analysed. The cows originated from different small farms, with low production intensity and pasture – mowing system, from south east part of Slovenia. This is a karst region where intensive farming is not possible.

Haematological variables; red blood cell count (RBC), haemoglobin concentration (Hb), mean corpuscular volume (MCV), haematocrit (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), number of platelets (PLT) and white blood cell count (WBC) were measured with a haematological counter ABC Vet (ABX, France). A differential white blood picture was also estimated by the examination of Giemsa stained blood smears. In blood serum samples biochemical variables; total serum protein (TSP), urea, calcium (Ca), inorganic phosphorus (iP), serum iron (Fe), magnesium (Mg), sodium (Na), potassium (K) and chlorine (Cl) were measured with the biochemical analyser Daytona (Randox). Beta carotene concentration was measured photometrically by the Yudkin method (Yudkin, 1941).

The data were processed using the statistical program SPSS (Ver 15.0) (SPSS, 2006). For the investigated variables the descriptive statistics were calculated. The individual data were checked regarding the reference intervals and the percentage of cows (samples) deviating from normal values for the investigated variables were calculated.

## RESULTS AND DISCUSSION

Regarding the anamnestic data problems with parasites occurred in 15 herds and in 14 herds there were fertility problems.

The mean values of investigated haematological variables were inside reference interval for cattle except the percentage of eosinophile granulocytes which was above the reference value (*Tables 1 and 2*). By checking individual data it was established that 55.6% of cows had the percentage of eosinophils above the reference value (*Table 3*). Eosinophilia could be associated with parasitic invasion or with allergic reactions (Jazbec, 1990; Kramer, 2006). In investigated cows it was most likely connected with endoparasites which corresponds with the anamnestic data although the parasite status was not directly determined in our study. In 16.7% of cows Hb concentration and in 31.0% PCV value was below the reference value. Low values of Hb and PCV indicate anaemia which can be attributed to parasitic invasion and/or some deficits in nutrition.

By checking the results of the biochemical examination it was established that in 45.2% of cows the concentration of TSP and in 28.8% the concentration of urea was below reference value. The concentration of TSP in cows is associated with proteins (amino-acid supply) available in the diet and also with animal's health status; globulins are increased by inflammation which can contribute to the increase in TSP concentration (Whitaker, 1997). The urea concentration in blood and milk is influenced by protein content and protein / energy ratio in the diet (Oltner and Wiktorsson, 1983). The results in investigated cows indicate insufficient protein supply and/or failure to provide a balanced diet.

**Table 1**

**Descriptive statistics of haematological variables**

Variable	n	Mean	SD	Min.	Max.
RBC $\times 10^{12}/L$	42	6.23	1.03	3.87	8.87
Hb g/L	42	103.2	17.5	58.0	142.0
MCV fL	42	49.6	5.6	37.0	62.0
PCV L/L	42	0.31	0.05	0.17	0.42
WBC $\times 10^9/L$	42	8.26	4.01	3.60	22.0
PLT $\times 10^9/L$	42	398.5	205.4	3	1042
MCH pg	38	16.65	1.96	11.80	20.90
MCHC g/L	38	335.7	9.9	295.0	355.0
Segmented neutrophils %	27	36.2	21.7	6.0	83.0
Eosinophils %	27	12.4	9.6	0.0	39.0
Basophils %	27	0.1	0.3	0.0	1.0
Lymphocytes %	27	49.3	18.0	11.0	84.0
Band neutrophils %	27	0.2	0.5	0.0	2.0
Monocytes %	27	1.7	2.6	0.0	11.0

**Table 2**

**Descriptive statistics of biochemical variables**

Variable	n	Mean	SD	Min.	Max.
TSP g/L	31	70.26	8.36	54.10	96.20
Urea mmol/L	51	2.90	1.84	0.47	10.03
Ca mmol/L	51	2.33	0.26	1.48	2.77
iP mmol/L	51	2.06	0.52	0.91	3.31
Na mmol/L	16	142.1	1.9	139.0	145.0
K mmol/L	16	5.66	0.79	4.69	7.29
Cl mmol/L	11	99.2	4.9	90.0	106.0
Fe $\mu$ mol/L	31	22.12	7.99	5.00	38.10
Mg mmol/L	26	1.16	0.26	0.72	1.77
Carotene gama%	19	573.4	313.3	138.0	1357.0

In 35.5% of cow serum iron was below the reference value. Adult cattle normally receive enough iron with forage (Jazbec, 1990) so it is not very likely that low serum iron was a consequence of iron deficit in the diet. Low serum iron was more possibly related to the parasitic invasion and chronic blood loss due to parasites.

In 31.6% of cows the concentration of carotene was below the normal value. Ruminants receive carotene predominately with green forage (grass) but in grass silage and in hay the content of carotene can decline especially when mistakes were made during the preparation (Jazbec, 1990). Deficit of carotene can have a negative influence on fertility (Rakes *et al.*, 1985; Dirksen *et al.*, 2006) what was reported in anamnestic data of some cows.

Some deviations were observed also regarding the mineral status; in 31.4% of cows the concentration of Ca was below the normal value, the concentration of iP was in 13.7% below and in 31.4% above the reference range, and in 43.7% the concentration of

K was above the reference value. Hyperkalaemia could be caused by potassium excess in the diet; it could be established also by acidosis (Carlson, 2002). Because the studied cows were from farms with low production intensity and they were fed predominantly with forage, it is more likely that hyperkalaemia was caused by potassium excess. In relation to nutrition the observed deviations in mineral status could be attributed to the inappropriate content of minerals in the diet and/or to unsuitable ratio between them. Periparturient period, age of animals and acidosis can also have an influence on the concentration of Ca and iP (Jazbec, 1990; Herdt, 2000). These deviations could have a negative influence on production, fertility, and health of cows.

**Table 3****Percentage of cows (samples) deviating from the reference values**

Variable	n	Below ref. value (%)	Above ref. value (%)	Reference range
RBC $\times 10^{12}/\text{L}$	42	9.5	4.8	5.0–8.5
Hb g/L	42	16.7	2.4	90.0–140.0
MCV fL	42	2.4	4.8	40–60
PCV L/L	42	31.0	4.8	0.28–0.38
WBC $\times 10^9/\text{L}$	42	14.3	16.7	5.0–10.0
PLT $\times 10^9/\text{L}$	42	14.3	4.8	200–800
MCH pg	38	5.3	0	14.0–24.0
MCHC g/L	38	0	2.6	260.0–350.0
Segmented neutrophils %	27	33.3	25.9	25–45
Eosinophils %	27	0	55.6	1–10
Basophils %	27	0	0	0–2
Lymphocytes %	27	33.3	18.5	45–65
Band neutrophils %	27	0	0	0–2
Monocytes %	27	0	3.7	0–8
TSP g/L	31	45.2	9.7	70.0–80.0
Urea mmol/L	51	28.8	3.8	1.66–6.66
Ca mmol/L	51	31.4	0	2.25–2.99
iP mmol/L	51	13.7	31.4	1.61–2.25
Na mmol/L	16	0	0	135–157
K mmol/L	16	0	43.7	4.2–5.8
Cl mmol/L	11	0	0	90.0–108.8
Fe $\mu\text{mol}/\text{L}$	31	35.5	0	21.0–45.0
Mg mmol/L	26	0	26.9	0.69–1.23
Carotene gama%	19	31.6	0	>400

Source: Jazbec, 1990

## CONCLUSIONS

The results of haematological and biochemical examinations indicate that the major problems in investigated cows were parasitic invasion and unbalanced diet, having a negative influence on health status, fertility and productivity of these cows. Therefore, if better care would be given to parasite control and to the composition of the diet the

production in such herds would be improved and more profitable. Data from the present study provide a rough assessment of the situation in small herds. For a more accurate assessment higher number of animals should be investigated and a precise anamnestic data about herds included.

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## Genetics evaluation for longevity in Slovenian Simmental cattle

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### ABSTRACT

*A maternal grandsire model (sire-mgs model) using survival analysis was used to estimate fixed and to predict random effects in genetic evaluation for longevity of Slovenian Simmental population. Longevity was described as length of productive life (LPL). The culling frequency increases with lactation duration with the culling peak at the lactation end. At the end of second lactation, most cows were culled, while the number of culled cows decreases in subsequent lactations. Most culled cows first calved at the age of 26 to 30 months and the relative culling risk increases with the increase of age at first calving. The cows that are breed in herds with decreasing size are more likely to be culled. Determined minimal differences in estimated breeding values between analysed birth years indicate that in the period from year 1965–1998 selection for longevity was not conducted. In the period from year 1998 to year 2004 relatively high differences between average estimated sires BV for longevity per birth year were observed which could not be taken as accurate presentation BV because of the small number of data per sire. As the genetic evaluation accuracy highly depends on ratio of censored and uncensored records and also on number of daughters per evaluated sire, further research with the purpose to detect the impact of censored records proportion on accuracy of genetic evaluation as well as the determination of number of daughters per sire sufficient for accurate evaluation is necessary.*

(Keywords: cattle, Slovenian Simmental, longevity, Weibull proportional hazards model)

### INTRODUCTION

Longevity is a trait with great impact on dairy production economy and is therefore of considerable importance in dairy cattle breeding programmes (Charffeddine *et al.*, 1996; Strandberg and Soelkner, 1996). With the increase of longevity, the proportion of mature cows that produce more milk increases. For example, Strandberg (1996) estimated that an increase in longevity from three to four lactations increases average milk yield per lactation and profit per year by 11 to 13%. In addition, improvement in longevity decreases replacement costs and somewhat increases selection intensity.

The aim of this study was to present the results of genetic evaluation for the length of productive life in Slovenian Simmental population using a Weibull proportional hazard model.

### Review of literature

There are several ways to implement longevity in the breeding goal, directly or indirectly. Direct longevity can be represented as the length of productive life (LPL) or

stayability. In cattle breeding LPL is usually defined as the elapsed time between the first calving and culling, while stayability is defined as a binary trait that measures cow survival (live or culled) at certain point in time. The use of LPL is preferred since stayability as a discrete trait provides less information. Unfortunately LPL, as well as stayability, can be quantified only after the cows are culled, though both approaches provide partial information when cow survives to the next “period” in life. Therefore, the information on the longevity of daughters of a sire becomes available with the increasing age of a sire. This inherently leads to the prolonged generation interval. Low heritability for longevity (Short and Lawlor, 1992; Vollema and Groen, 1996) induces unreliable estimation of breeding values (BV) based on the information of parents or grandparents.

Due to a long generation interval, breeding programmes also include indirect measure of longevity via correlated traits such as fertility, health, and conformation traits (Burnside et al., 1984). Additional gain is due to the fact that the data on these indirect traits can be collected relatively early in the life of a cow. Nonetheless, both representations of longevity (direct and indirect) have a merit in a modern breeding goal (Essl, 1998).

Analysis of indirect representations of longevity is to a large extent done with a standard linear model based on the Gaussian (normal) distribution. Specific approach is needed for a proper analysis of the LPL, due to the presence of live animals at the time of analysis (censored records) and changes in culling criteria over the productive life of cows (time-dependent effects) (Ducrocq et al., 1988). Exclusion of censored records from the analysis, or treating them as uncensored would lead to biased results (Ducrocq, 1994). Additionally, the mode of relationship between longevity and its effects is rather multiplicative than additive (Ducrocq et al., 1988). Survival analysis can handle this kind of data. In the last years several countries introduced direct longevity in the routine genetic evaluation of cattle and most of them use the Weibull proportional hazard model (INTERBULL, 2009), which represents a class of models in the field of survival analysis. Other statistical approaches (models) can also be used, but proportional hazard model have better properties (Caraviello et al., 2004; Jamrozik et al., 2008).

## MATERIALS AND METHODS

Raw data for 142,989 Slovenian Simmental cows born from 1980 to 2008 were provided by the Agricultural Institute of Slovenia. In order to use old data but to avoid modelling the data up to the year 1991, the truncation date was set at January 1<sup>st</sup> 1991. On the other side, the date of last data collection was January 29<sup>th</sup> 2010. For cows alive at that time longevity was treated as right censored. Longevity was defined as the length of productive life (LPL) and was calculated as the number of days from the first calving to culling (uncensored/complete records) or to the moment of data collection (incomplete/censored records). The LPL of cows surviving beyond the sixth lactation was also censored in order to avoid the effect of preferential treatment and to focus on early culling in the life of a cow. Cows with missing or inconsistent data within the defined limits were removed (51,412 cows): culling before the date of truncation, calving date after the date of culling, no information for 600 days after calving, missing data for the first three lactations, daughters of sires with less than 20 daughters, and missing covariate or factor data.

Altogether LPL for 125,468 cows from 6791 herds were used in the analysis. Cows in the analysis were daughters of 686 sires. MGS pedigree included 1903 sires. Cows were on average culled between 3<sup>rd</sup> and 4<sup>th</sup> lactation, which amounted to 1212 day of productive life. Percentage of censored records was 44.9%.

Weibull proportional hazards model was used for the analysis of LPL. This model is built upon the Weibull distribution, whose density (1) and hazard (2) function for the i-th record are:

$$f(t_i | \lambda, \rho) = \lambda \rho (\lambda t_i)^{\rho-1} \exp(-(\lambda t_i)^\rho) \quad (1)$$

$$h(t_i | \lambda, \rho) = \lambda \rho (\lambda t_i)^\rho \quad (2)$$

where  $\lambda$  (scale) and  $\rho$  (shape) are strictly positive parameters. In proportional hazard model it is assumed that the baseline hazard function changes proportionally with change in covariate(s) or factor levels. For the analysis of LPL the hazard function was modelled as:

$$h(t_{ijklmop} | \lambda, \rho, \text{else}) = h_0(t_{ijklmop} | \lambda, \rho) \exp(c_i + l_j + y_k + h_l + d_m + s_n + 1/2 s_o) \quad (3)$$

where:

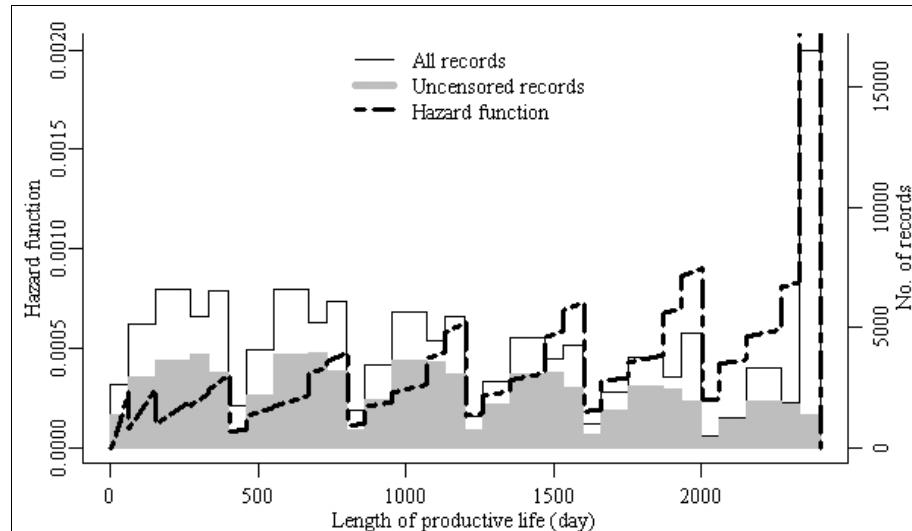
- the hazard function of culling the p-th cow given all parameters,
- the baseline Weibull hazard function (2),
- the time-independent effect of the i-th age at the first calving: 0 (unknown) and from 19 to 50 months,
- the time-dependent effect of the j-th lactation stage (1–60 days, 61–150 days, 151–270 days, 271-days till drying, and dry period) within parity – altogether 30 levels,
- the time-dependent effect of the k-th season defined as year (1990–2009),
- the time-dependent effect of the l-th herd (3891 levels),
- the time-dependent effect of the m-th herd size deviation in comparison to previous year ( $\leq -70\%$ ,  $(-70\%, -40\%]$ ,  $(-40\%, -10\%]$ ,  $(-10\%, 10\%]$ ,  $(10\%, 40\%]$ ,  $(40\%, 70\%]$ , and  $>70\%$ ),
- the time-independent effects of the n-th sire and the o-th maternal grandsire (onwards both effects are termed sire effect) of the p-th cow.

Levels of time-dependent effects changed with cow “status” changes in time, while levels for time-independent effects were constant over whole lifetime of a cow. Altogether, there were 2,644,036 elementary records. Herd and sire effects were modelled hierarchically: log-gamma distribution for herd effect and multivariate normal for sire effect with additive genetic covariance matrix among sires.

Data processing was done with SAS software package (SAS Institute, 2000), while Survival Kit version 3.10 (Ducrocq and Soelkner, 1998) was used for modelling and parameter estimation. In the first step a series of log-likelihood ratio tests were performed for effects that were not modelled hierarchically – importance of each effect was tested as a comparison between the full model and the model where the effect under testing was excluded. In the next step herd and sire effect were added to the model to obtain estimates for all model parameters.

## RESULTS AND DISCUSSION

All effects included in the model were highly significant ( $P < 0.001$ ) which is not surprising given the size of data set and the previous knowledge of effect importance for LPL. Estimates for relative risk of culling according to the effect of lactation stage within parity are shown in *Figure 1*. It could be noticed that one year after calvings that is at the end of lactation periods cullings are most frequent.

**Figure 1****Relative risk of culling and number of records by stage of lactation within parity**

The intervals between the culling peaks are about 400 days which are equal to average calving interval in Slovenian Holstein cows. In respect to parity, most cows were culled at the end of second lactation, then at the end of third, first and fourth lactation. The number of culled cows decreases in subsequent lactations. Line at the figure that connects estimates of effect classes shows culling frequency during each lactation. In all lactations, with exception of the first one, culling frequency increases with lactation duration with culling peak at the lactation end.

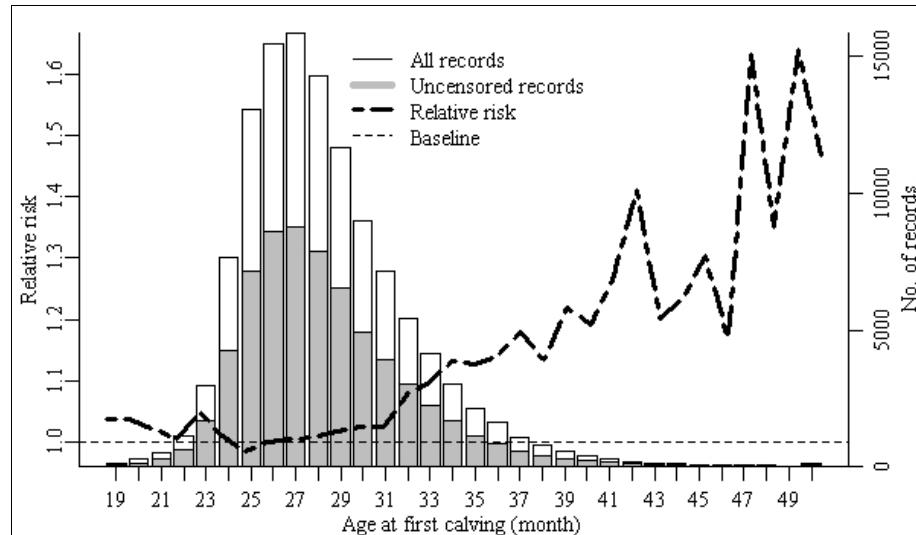
*Vukasinovic et al.* (1997) determined that the relative culling rate is larger during the first lactation than during later lactations. They also determined that within each lactation, the relative culling rate varies significantly that is a cow finishing a lactation is at much higher risk of being culled than an identical cow in early or midlactation, because the dry period is the period of the most intensive selection in dairy herds. They also noticed that the risk of being culled increases up to 2 months after calving, which might be due to a higher incidence of health disorders during early lactation.

According to age at first calving, the proportion of culled cows increases till the age of 27 months, after what decrease is determined. Most culled cows first calved at age 25 to 29 months (*Figure 2*). Relative culling risk increases with increase of age at first calving. *Vollema and Groen* (1998) determined that effect of age at first calving on culling risk was significant ( $P<0.001$ ) and positive indicating that cows that are older at first calving have a higher risk of being culled. *Ducrocq et al.* (1988) and *Ducrocq* (1994) in statistical analysis of length of productive life for dairy cows determined that the effect of age at first calving was not significant. *Rogers et al.* (1991) in analysis of survival of Jersey breed found that productive life decreased with age at first calving. *Syrstad* (1979) showed that survival rates of cows declined dramatically over 34 months of age at first calving and concluded that an intermediate age at first calving was associated with the highest LPL. *Vukasinovic et al.* (1997) tested the significance of the explanatory variables using a likelihood ratio test for large samples and stated that the

change in log likelihood that was associated with the age at first calving was very small compared with that of the other effects analysed in their study. Therefore, they considered this effect as unimportant.

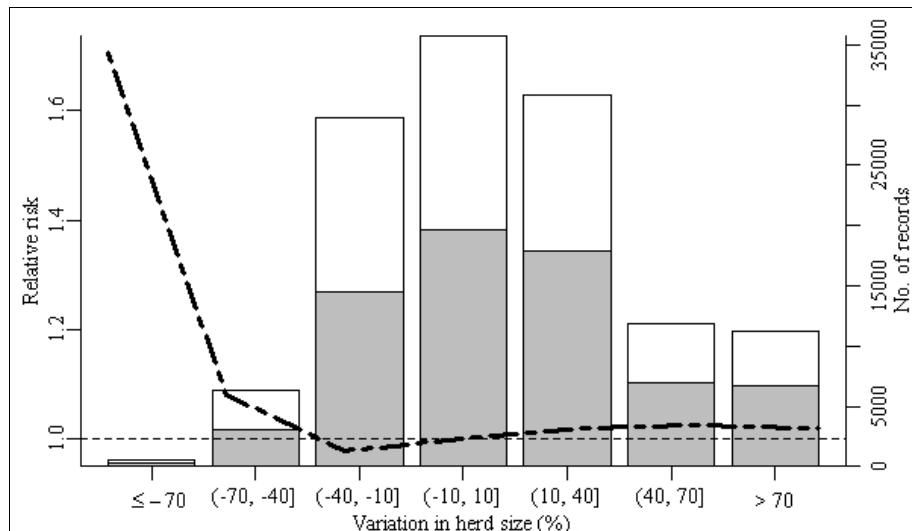
**Figure 2**

**Relative risk of culling and number of records by age at the first calving**



According to classes of deviation in herd size across years, highest proportions of culled cows were determined in 4. class [40%, 70%], while the highest culling risk was noticed in 1. class [min, -70%] meaning, as expected, that the cows that are breed in herds with decreasing size are more likely to be culled (Figure 3). *Weigel et al.* (2002) calculated the relative culling risk of high producing (top 20%) and low producing (bottom 20%) cows relative to average cows in the same herd in regard to changes in herd size. They determined that, before herd expansion, low producing cows were 4.2 times more likely to be culled than average cows, while high producing cows were only 0.5 times as likely to be culled as average cows. After herd expansion, the relative risk for low producing cows dropped to 2.6 times that of average cows, and the risk for high producing cows increased to 0.7 times that of average cows. This indicate that in expanding herds more high producing cows are culled due to involuntary culling and fewer low producing cows could be culled due to low milk production. Same authors also reported that relative risk of involuntary culling of high producing cows (as compared with average cows) in expanding Wisconsin herds depends of the herd size that is in larger herds ( $>150$  cows) is higher (0.71) than in smaller herds ( $<150$  cows; 0.62).

Based on the used statistical model, sire variance in amount of 0.030, and herd variance in amount of 0.199 were estimated. Estimated heritability value in amount of 0.098 was similar to values estimated in the Nederlands (INTERBULL, 2009) and joint evaluation for Austria and Germany (Fuerst and Egger-Danner, 2002 and relative low in comparison of this parameter estimated in Switzerland (0.198) (Vukasinovic et al., 2001).

**Figure 3****Relative risk of culling and number of records by levels of variation in herd size**

The genetic evaluation accuracy highly depends on ratio of censored and uncensored records. As the proportion of censored records decreases, the evaluation accuracy increases. Also, it is necessary to have sufficient number of daughters per sire. *Vukasinovic et al.* (1997) stated that more than 30 to 40% of censored records would lead to inaccurate results. Same authors reported that small number of daughters per sire without any or with only few uncensored records would bias the sires ranking according to estimated transmitting abilities (ETA). *Egger-Danner et al.* (1993) in their study compared the ranking of sires according to ETA from the full data file without censored records and from truncated data with a different proportion of censored records and observed that rank correlations between ETA on the full data file and on the censored data were lower as the proportion of censoring increased.

Further research with purpose of detection of the impact of censored records proportion on accuracy of genetic evaluation as well as the determination of number of daughters per sire sufficient for accurate evaluation is necessary.

## CONCLUSIONS

Appliance of survival analysis provide usage of uncensored and censored records what makes this method adequate to use for analysis of productive life data. Based on the conducted research it could be concluded that all effects included in used statistical model (time-independent fixed effect of time from 1st calving to the culling or to the moment of data collection or till the end of sixth lactation; time-independent fixed effect of lactation stage within parity; time-independent fixed effect of age at first calving; time-dependent fixed effect of year; time-dependent fixed effects of herd size class; time-dependent random effect of herd and time-independent random genetic effect of sire) have significant effect on LPL. The most important effects on LPL are lactation stage and parity, age at first calving, as well as the effect of changes in herd size across years.

The culling frequency increases with lactation duration with culling peak at the lactation end. At the end of second lactation, most cows were culled, while the number of culled cows decreases in subsequent lactations. Most culled cows first calved at the age of 25 to 29 months and the relative culling risk increase with increase of age at first calving. The cows that are bred in herds with decreasing size are more likely to be culled. Based on used statistical model, sire variance in amount of 0.030, herd variance in amount of 0.199 as well as heritability value in amount of 0.098 were estimated.

The genetic evaluation accuracy highly depends on ratio of censored and uncensored records and also on number of daughters per evaluated sire, therefore further research with purpose of detection of the impact of censored records proportion on accuracy of genetic evaluation as well as the determination of number of daughters per sire sufficient for accurate evaluation is necessary.

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## Pedigree analysis of Burlina cattle population

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### ABSTRACT

*The aim of this study was to characterize the pedigree of Burlina cattle breed. The file was supplied by the Breeders Association of Treviso province (Italy) and included 2303 animals. About 79% of individuals had both parents known. The additive genetic relationship ( $f$ ) among animals and the coefficient of inbreeding ( $F$ ) were calculated as well as the number of generations and pedigree completeness index (PCI). The PCI increased over the studied period and individuals born in 2009 had an average value of 90% considering 2 generations and 69% considering 5 generations. The additive genetic relationship increased across years, reached a value of 1.95% in 2004 and then decreased slightly. Coefficient of inbreeding was low in 1990s and increased rapidly afterwards; in 2009 the  $F$  value was 4.81%. The monitoring of  $f$  and its use as weighting factor for selecting sires and dams of future bulls will be used to control inbreeding levels within the population.*

(Keywords: additive genetic relationship, Burlina breed, inbreeding, pedigree completeness)

### INTRODUCTION

Burlina is a native cattle breed reared in north-east Italy. The number of animals consistently decreased up to the end of 1970s, mainly because of the progressive substitution of this population with the more productive Holstein Friesian (CNR, 1983; *Del Bo et al.*, 2001). At the beginning of 1980s, the Burlina was enrolled in the Italian Herd Book of local breeds and pedigree information was registered more accurately.

An important step for the conservation of a native animal genetic resource is to assess the magnitude of its genetic variability (Wright, 1922; *Del Bo et al.*, 2001; *Dalvit et al.*, 2008); this can be achieved through the additive genetic relationship among animals and the coefficient of inbreeding, which are simply calculated using pedigree information as proposed by Wright (1922). However, problems in the reliability of these coefficients may arise if individual ancestry is not recorded back to a common base population (*VanRaden*, 1992).

The best strategy to manage a population under conservation is to optimize contributions of parents by minimizing the global additive genetic relationship weighted by those contributions (*Meuwissen and Sonesson*, 1998; *Fernández et al.*, 2005; *Sørensen et al.*, 2008). This is particularly true for small populations at risk of genetic erosion. The aim of this study was to characterize the pedigree of Burlina breed, particularly in relation to the completeness of the pedigree, the additive genetic relationship among animals and the coefficient of inbreeding.

### MATERIALS AND METHODS

The pedigree file was supplied by the Breeders Association of Treviso province (Italy) and included 2303 animals. About 79% and 14% of individuals had both parents known

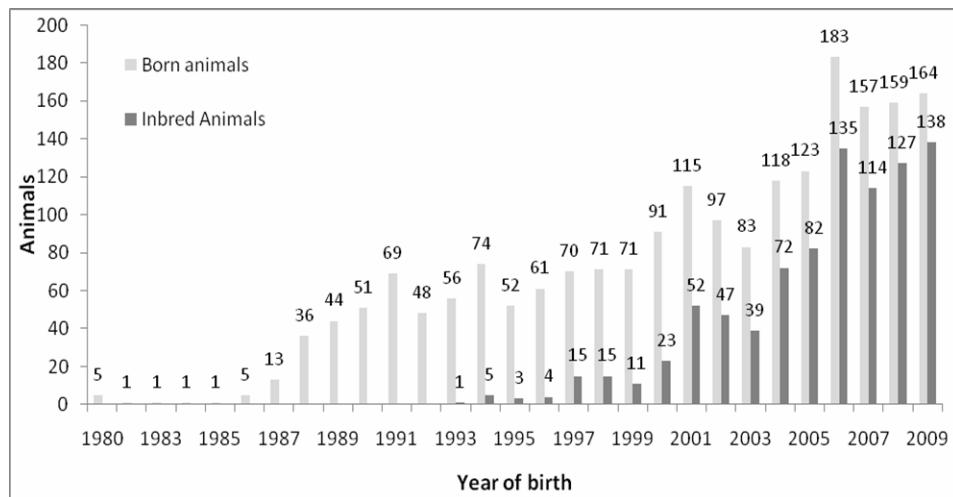
and unknown, respectively, while the remaining 7% had only one of the two parents unknown. The base population was assumed to be born in 1980. For individuals with both parents known, the year of birth ranged from 1986 to 2009. The additive genetic relationship ( $f$ ) and the coefficient of inbreeding ( $F$ ) were calculated using the INBREED procedure of SAS (2006), and number of generations and pedigree completeness index (PCI) were obtained through EVA software package (Berg et al., 2007).

## RESULTS AND DISCUSSION

Figure 1 displays the distribution of animals by year of birth. Programs to safeguard Burlina breed were set up in 1980s (Bittante et al., 1992) and the number of individuals gradually increased during the last 20 years. At the beginning of the conservation program, bulls of the local breed were mated to pure and crossbred Burlina cows (Bittante et al., 1992) and then backcross to Burlina was practiced. In fact, there were not inbred animals in 1980s while in the 1990s the number of inbred individuals increased. About 81% of female and 88% of male calves born in 2009 were inbred, with an average  $F$  of  $5.49 \pm 5.21$  and  $6.02 \pm 4.97$ , respectively (data not shown).

**Figure 1**

**Distribution of animals by year of birth**



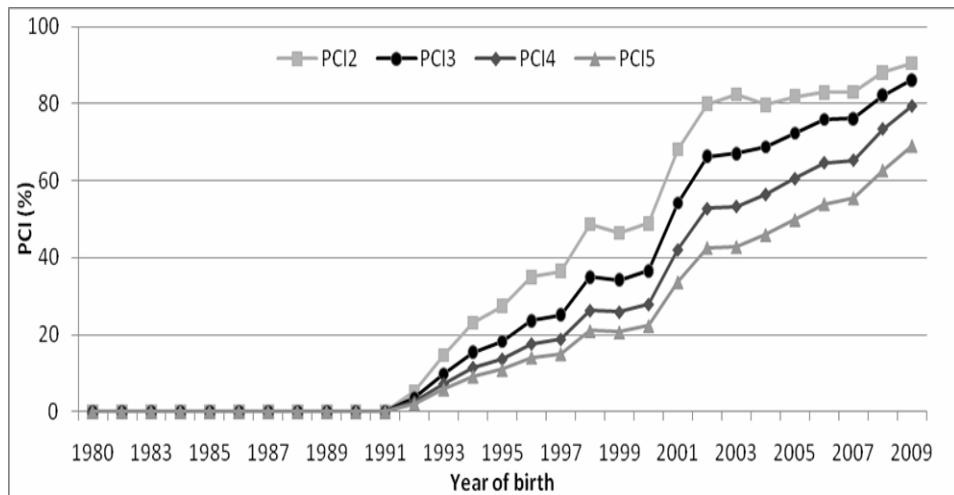
The PCI is an important index to assess the quality of pedigree and investigates the depth of pedigree itself; it is proportionally inversely to the number of generations considered. For example, animals born in 2009 had an average PCI of 90% considering 2 generations and 69% considering 5 generations (Figure 2). An interesting approach is to evaluate how many animals had a PCI equal to 100%. In 2009, 74% of individuals had 2 generations completely known, but this percentage was only 23% when at least 3 generations were considered (Figure 2).

In this situation, it is more informative to look at the average  $f$  value over the years (Figure 3). The additive genetic relationship among animals increased across years; in 1980s it ranged from 0.00 to 0.51, from 1990 to 2004 the rate strongly increased (0.46 to

1.95%) and then slightly decreased. Animals born in 2009 had an average  $f$  of 1.86%. As expected, the trend of inbreeding resembled that of additive genetic relationship, i.e., it was low in 1990s and increased rapidly afterwards with a value of 4.81% in 2009. *Figure 4* shows the average  $f$  by year of birth of sires. In general, the  $f$  value was constant across the years, and ranged from 1.12 to 2.15%.

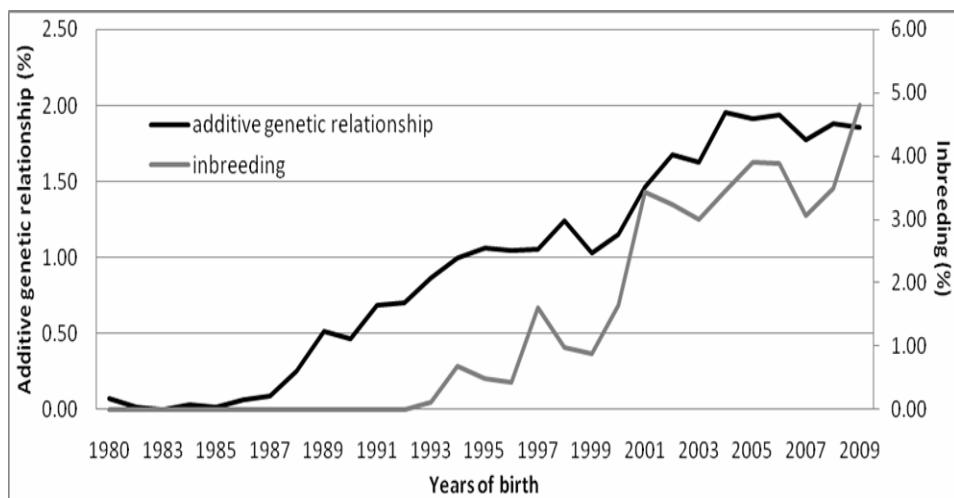
**Figure 2**

**Pedigree completeness index (PCI, %) by year of birth considering 2 (PCI2), 3 (PCI3), 4 (PCI4) and 5 (PCI5) generations**



**Figure 3**

**Average additive genetic relationship (%) and inbreeding (%) by year of birth**



**Figure 4**

**Average additive genetic relationship (%) of sires by year of birth**



**CONCLUSIONS**

The number of inbred animals and the coefficient of inbreeding have increased over years. The same trend has been found for the additive genetic relationship among animals, but with a plateau during the last 5 years. The Burlina breed has an informative pedigree that could be used for further analyses. The monitoring of additive genetic relationship and its use as weighting factor for selecting sires and dams of future bulls as proposed by *Berg et al.* (2007) seems to be an important perspective to control inbreeding level and maintain genetic variability within the population.

**ACKNOWLEDGEMENTS**

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## **SECTION 4**

### **BUFFALO, HORSE AND SHEEP BREEDING**





## Molecular characterization of k-casein allelic variants in Bulgarian buffalo breed

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### ABSTRACT

*This study aimed to analyze the genetic polymorphism at the k-CN (CSN3) locus in the Murrah Bulgarian buffalo breed. A 221 bp fragment of exon IV of CSN3 gene was amplified and sequenced to detect polymorphisms in 215 animals. Genotyping, by means of sequence alignments, revealed the presence of one polymorphism at codon 135 that occurs with a substitution of a Thr (ACC) of the A genetic variant for an Ile (ATC) of the B genetic variant, while codon 148 resulted monomorphic for the presence of an Ala residue (GCT). Additionally a silent mutation Thr-Thr (ACC/ACT) occurs at 136 codon position. Genotypes frequencies at codon 135 were 0.56 (AA), 0.40 (AB) and 0.04 (BB) being the gene frequencies 0.76 and 0.24 for the A and B allele respectively. No significant deviation from Hardy-Weinberg Equilibrium was observed.*

(Keywords: k-casein, genotyping, Murrah Bulgarian buffalo)

### INTRODUCTION

Caseins are a family of milk proteins that approximately constitute 80% of total lactoproteins (Otaviano *et al.*, 2005). They are largely present under micelle form, which consists of four phosphorylated fractions:  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$ , k-casein. Post-translational modifications together with the presence of genetic variants are the factors which determine casein heterogeneity (Ferranti *et al.*, 1998). Casein genes have been studied intensively because, in domestic animals, their polymorphism has been associated to difference in milk composition, processing, quality and also yield characteristics.

Genotyping of milk proteins is extremely important for selection practice to improve industrial milk production. To date nine variants have been found in cattle for k-CN gene: A, B, C, E, F, G, H, A1 and J (Dogru *et al.*, 2009). A and B variants are the most common in cosmopolitan and local cattle breeds (Chessa *et al.*, 2007; Pacini *et al.*, 2008). The variant B has a significant direct effect on protein content, and on milk yield (Ganagaraj *et al.*, 2008): the milk from BB genotypes results in shorter rennet coagulation time, formation of a firmer curd and a greater cheese yield than milk from AA genotype (Otaviano *et al.*, 2005; Comin *et al.*, 2008). The k-CN A allele has a threonine (ACC) aminoacid at position 136 and an aspartic acid (GAT) at position 148 (Farrell *et al.*, 2004; Chianese *et al.*, 2009). In the k-CN B variant isoleucine (ATC) and alanine (GCT) substitute the aforementioned threonine and aspartic acid residues (GenBank: CAP12622.1). The aim of this work was to genetically characterize the k-CN gene in Murrah Bulgarian buffalo breed in order to investigate the different allelic variants present in the population.

## MATERIALS AND METHODS

Ear-punch samples were collected from 215 Bulgarian Murrah buffaloes. DNA was purified using Maxwell16® station and the Maxwell16®Tissue DNA Purification Kit (Promega). DNA was precipitated o/n with three volumes of 70% cold EtOH, 0.1 M sodium acetate (pH 5.2) and 2 µl of glycogen 2 mg/ml, pellet was then washed twice in 70% cold EtOH and resuspended in 50 µl of ddH<sub>2</sub>O. DNA concentration was estimated using Qubit® fluorometer (Invitrogen). A 221 bp fragment of exon IV of the CSN3 gene was amplified using primers: Bub-CSN3-f 5'-TGCCAAGCCCAGCCAATCACC-3' and Bub-CSN3-r 5'-CGACGGTTGAAGTAACCTGGGCTG-3'. 40 ng of DNA were used for PCR amplification using 0.20 µM of each primer, 1X HF-Buffer (Finnzymes), 0.2 mM dNTPs and 0.2 U Phusion-HF DNA polymerase (Finnzymes). The PCR conditions used were: initial denaturation at 98 °C for 30 s followed by 40 cycles of 98 °C for 7 sec, 64 °C for 15 s and 72 °C for 20 s, with a final extension step at 72 °C for 7 min. PCR products were purified with Agencourt AMPure Purification System (Beckman Coulter) and sequencing reaction was performed using GenomeLab™ DTCS Quick Start Kit for Dye Terminator Cycle Sequencing following manufacturer's instructions. The Agencourt CleanSEQ Purification System (Beckman Coulter) was used for the purification of sequencing products. Sequencing was performed on a CEQ8000 Genetic Analysis System (Beckman Coulter) and single-nucleotide polymorphism (SNP) was performed with Genetic Analysis Software v.9.00 (Beckman Coulter). Descriptive statistics were calculated using Genepop v.4 (Rousset, 2007).

## RESULTS AND DISCUSSION

A 221 bp fragment of exon IV of the CSN3 gene was amplified and sequenced in 215 buffaloes belonging to Murrah Bulgarian breed. Sequences were aligned and analyzed using BioEdit v. 7.0.5 (Hall, 1999) and a substitution of an Ile residue for Thr at 135 codon position was detected. A silent mutation at Thr<sup>136</sup> was also observed (ACC/ACT) while no genetic variant of Ala<sup>148</sup> was detected (*Table 1*).

**Table 1**

**Positions and amino acid differences in genetic variants of *k*-casein in cattle and buffalo**

	<b>Position and amino acid in the protein</b>		
	135	136	148
k-casein ( <i>Bos taurus</i> )	Thr	Thr (Ile)	Asp (Ala)
k-casein ( <i>Bubalus bubalis</i> )	Thr (Ile)	Thr	Ala

Between brackets are indicated the substitutions in cattle for the B allelic variant according to Farrell et al., 2004. The B variant observed in this study in buffalo is indicated in *italics*.

Mitra et al. (1998) reported the same amino acid substitution T135I of the *k*-CN gene in buffalo defined as B variant. Genotypic frequencies were 0.56, 0.40 and 0.04 for AA, AB and BB genotypes and gene frequencies of allele A and B were 0.76 and 0.24 respectively. Deviation between observed genotypic frequencies and those expected under Hardy-Weinberg equilibrium were not significant (P>0.05) suggesting that the Murrah Bulgarian population is in equilibrium at the CSN3 locus.

Recent studies in buffaloes used a PCR-RFLP approach with restriction enzymes *Hind*III, *Hinf*I and *Taq*I (Abbasi *et al.*, 2009 and Abdel Dayem *et al.*, 2009). Such approach can detect polymorphism in cattle where k-CN A and B variants differ for T136I and D148A but it could not detect the different genetic variants in our buffalo population because cattle sequence variations are not conserved. These findings demonstrate the importance of the direct sequencing approach to detect k-CN polymorphisms in buffaloes. Since k-CN variant B is associated with higher fat, protein, and casein content in cattle milk and it has a significant influence on cheese making properties and superior rennet coagulation properties in comparison to A variant (Heck *et al.*, 2009), further studies are required in order to confirm such association also in buffalo to eventually implement, in a selection program scheme, a MAS (Marker Assisted Selection) approach based on CSN3 marker.

## CONCLUSIONS

A portion of the buffalo CSN3 locus was successfully amplified and a polymorphism was observed at 135 codon position allowing the detection of A and B allelic variants. These results could be achieved with the use of direct sequencing approach while no polymorphism could be detected using PCR-RFLP technique implemented for k-CN genotyping in cattle.

Homozygote BB individuals result in lower frequencies than AA and AB genotypes but the gene frequency of the B allele is reasonably high to evaluate an effective strategy to increase this favorable genotypes. Moreover a study on functional aspects of the different genetic variant could be performed in buffaloes to confirm a positive effect of the B allele known in other livestock species.

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## Preliminary characterization of coagulation properties of buffalo milk in Veneto region

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### ABSTRACT

*This study is a preliminary contribution to the characterization of milk production from buffaloes reared in Veneto region and examines the relationships between milk coagulation properties (MCP) and milk composition traits. A total of 66 buffaloes were sampled during the morning milking and analyzed within 3 h from collection. Measures of MCP were obtained using a Formagraph whereas milk composition traits were assessed through a MilkoScan FT120. Significant relationships were found between production traits and MCP. Rennet coagulation time seems to be related mainly to milk yield and casein content whereas curd firmness to protein percentage, casein percentage and acidity. With respect to curd firming time, a significant relationship has been found with casein content and acidity. Results allowed a preliminary characterization of buffalo milk traits in northeast Italy. Further studies are needed to better investigate the variability of MCP and the relationships between MCP and cheese yield.*

(Keywords: buffalo, milk coagulation properties, milk quality traits)

### INTRODUCTION

In Italy, buffaloes (*Bubalus bubalis*) are used as dairy animals, because they have been selected for milk production and have acquired specific traits characteristic of the actual “Italian Mediterranean buffalo”. The use of buffaloes is steadily increasing in terms of both number of animals and farms also in the northern part of Italy because of the economic returns coming from the transformation of milk in the traditional Mozzarella cheese and other innovative products (Addeo *et al.*, 2007). Therefore, buffaloes may represent a potential tool for dairy farms to differentiate products and increase competitiveness in the market.

Milk coagulation properties (MCP) are of great relevance for cheese production, mainly because of their role in cheese-making (Aleandri *et al.*, 1989; Wedholm *et al.*, 2006; De Marchi *et al.*, 2008). Good reactivity to rennet, high curd firming capacity, good syneresis ability and whey drainage are crucial features of milk transformed into cheese. Assessment of MCP can be performed through computerized renneting meter (Annibaldi *et al.*, 1977; Zannoni and Annibaldi, 1981), providing measures of rennet coagulation time (RCT, min), curd firmness ( $a_{30}$ , mm) and curd firming time ( $k_{20}$ , min). Measurement of MCP is of special relevance for cheese manufacturing as cheese yield tends to increase with decreased RCT and increased  $a_{30}$ .

Previous studies (Zicarelli *et al.*, 2001; Potena *et al.*, 2001a; Potena *et al.*, 2001b) investigated the variability of milk quality traits in buffaloes and their relationships with

MCP in the southern Italy; however, such relationships are not yet fully explained. This study is a preliminary contribution to the characterization of milk production of buffaloes in Veneto region and examines the relationships between MCP and milk composition traits.

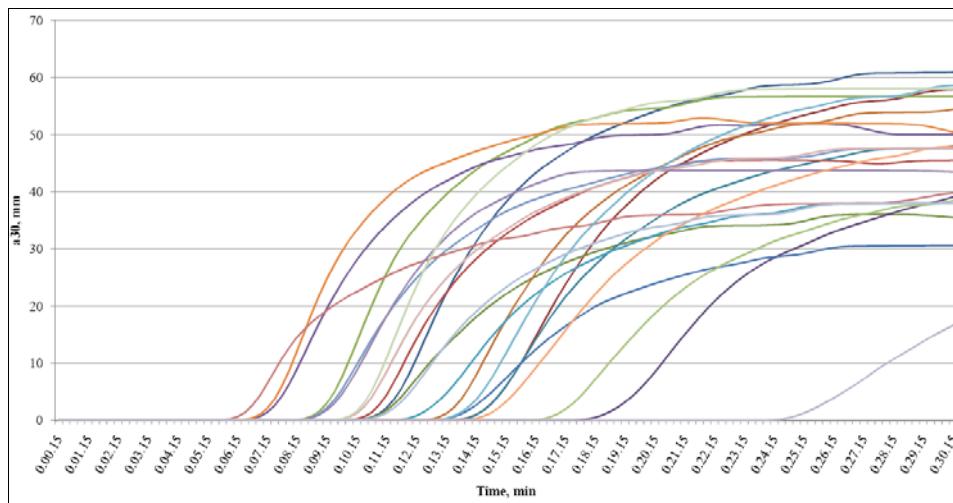
## MATERIALS AND METHODS

A total of 66 buffaloes, reared in one herd located in Veneto region, were sampled once in February 2010. Milk samples (50 mL) were collected during the morning milking, stored in portable refrigerators (4 °C), transferred to the milk quality lab of the Department of Animal Science (Padova, Italy) and analyzed within 3 h from collection.

Ten mL of milk were heated to 35 °C; once the temperature was reached, 200 µL of rennet (Hansen standard 190 with 63% of chymosin and 37% of pepsin, Pacovis Amrein AG, Bern, Switzerland) diluted 1.6% in distilled water were added to milk. Measurement of MCP, determined by a Formagraph (Foss), ended within 31 min after the addition of the clotting enzyme. Measured traits were RCT (the time interval in minutes from the addition of the clotting enzyme to the beginning of coagulation),  $k_{20}$  (the time in minutes required to achieve 20 mm of firmness), and a 30 (the width in millimeters of the diagram 31 min after rennet addition, i.e., the firmness of the curd; *Figure 1*). The remaining amount of milk (40 mL) was used to determine fat and protein contents and titratable acidity through the MilkoScan FT120 (Foss). All samples were warmed up to 35 °C using a water bath and mixed gently prior to spectra recording at this temperature. The analysis is based on the FTIR measuring principle, respecting IDF and AOAC standards. Pearson product-moment correlations were obtained through the CORR procedure of SAS and were used to evaluate the relationships among milk attributes and MCP.

**Figure 1**

**Coagulation process of buffalo milk samples**



## RESULTS AND DISCUSSION

Descriptive statistics of MCP, milk yield and quality traits are reported in *Table 1*. Milk yield averaged 8.04 kg/d. Protein, casein and fat contents were 4.73, 3.86 and 8.24%, respectively and showed large variability. Milk yield as well as milk attributes are comparable to the average of buffaloes registered in the National Herdbook (ANASB, 2009). Measure of fat content was more variable compared to other components; however, the value is in agreement with that reported by *Pilla and Moioli* (1992) and *Tiezzi et al.* (2009).

**Table 1**

### Descriptive statistics of milk yield, quality traits and coagulation properties in buffalo cows

Trait <sup>1</sup>	N	Mean	SD	Minimum	Maximum
DIM, d	58	110	56	13	218
MY, kg/d	60	8.04	2.88	2.70	17.80
Protein, %	63	4.73	0.48	3.68	5.97
Casein, %	63	3.86	0.36	2.99	4.50
Fat, %	63	8.24	1.61	4.72	12.45
TA, °SH/100	63	9.06	1.47	5.88	12.97
RCT, min	63	12.51	4.45	6.00	25.45
$k_{20}$ , min	61	2.71	0.98	1.45	6.30
$a_{30}$ , mm	63	44.10	11.56	10.60	65.00

<sup>1</sup>DIM: days in milk, MY: milk yield, TA: titratable acidity, RCT: rennet coagulation time,  $k_{20}$ : curd firming time,  $a_{30}$ : curd firmness

Averages for RCT,  $k_{20}$  and  $a_{30}$  were 12.51 min, 2.71 min and 44.10 mm, respectively. These measures are close to recommended values in practical cheese making by *Zannoni and Annibaldi* (1981) and are better than those frequently found in dairy cows (*Cassandro et al.*, 2008). Values of RCT,  $k_{20}$  and  $a_{30}$  observed in this study are indicative of faster coagulation rates and better results of the curd firming process than those reported by *Ariota et al.* (2007) for individual milk samples of buffaloes. However, *Bartocci et al.* (2002) reported values of  $a_{30}$  higher than our results, ranging from 52.48 to 55.59 mm.

Pearson's correlations among milk yield, quality traits and coagulation properties are in *Table 2*. As expected, estimates among MCP traits were high and ranged from 0.66 to 0.82 in absolute value. These results were expected as MCP describe consecutive steps of the milk coagulation process. If milk takes a short time to coagulate, it leaves more time for curd firming and has better coagulation ability in general; thus, the final curd will be firmer. Conversely, if milk takes a long time to coagulate, the curd will have less time to firm and it will be weaker. Favourable MCP were associated with high casein content (-0.25 and 0.71 with RCT and  $a_{30}$ , respectively). The strong correlation of  $a_{30}$  with casein confirms the key role of casein in milk coagulation process. The importance of casein in increasing curd firmness of cow's milk has been reported by several authors (*Pagnacco and Caroli*, 1987; *Summer et al.*, 1999). The relationship between MCP and protein was slightly lower than casein, however it is worth noting that  $a_{30}$  and protein are strongly related. Correlations of acidity with  $k_{20}$  and  $a_{30}$  were

moderate (-0.52 and 0.59 with RCT and  $a_{30}$ , respectively), while no relationship has been found between acidity and RCT. This result is in disagreement with estimates reported in literature on cows which usually highlighted the strong relationship between acidity and RCT. The correlation of MCP with fat was weak and not significantly different from zero (except for  $a_{30}$ ), in accordance with values reported by *Lindström et al.* (1984). It appears that the amount of fat does not influence the coagulum strength nor it is able to be completely retained during the cheese making process. This result appears to be consistent with the work of *Aleandri et al.* (1989) who found that the efficiency of fat recovery (retention of fat in the curd) increased with the protein to fat ratio. The relationship between milk yield and RCT was moderate and favourable; this result is not consistent with other findings on bovine milk (*Cassandro et al.*, 2008).

**Table 2****Correlations among milk yield, quality traits and coagulation properties in buffalo cows**

Trait	MY	Protein	Casein	Fat	TA	RCT	$k_{20}$	$a_{30}$
MY, kg/d	-	-0.25*	-0.08 <sup>ns</sup>	-0.30*	-0.18 <sup>ns</sup>	-0.52***	-0.24 <sup>ns</sup>	0.27*
Protein, %		-	0.87***	0.57***	0.90***	-0.04*	-0.44***	0.58***
Casein, %			-	0.73***	0.85***	-0.25*	-0.56***	0.71***
Fat, %				-	0.60***	0.16 <sup>ns</sup>	-0.20 <sup>ns</sup>	0.30*
TA, °SH/100					-	-0.11 <sup>ns</sup>	-0.52***	0.59***
RCT, min						-	0.74***	-0.66***
$k_{20}$ , min							-	-0.82***
$a_{30}$ , mm								-

MY: milk yield; TA: titratable acidity; RCT: rennet coagulation time;  $k_{20}$ : curd firming time;  $a_{30}$ : curd firmness; <sup>ns</sup> not significant; \*  $P<0.05$ ; \*\*  $P<0.01$ ; \*\*\*  $P<0.001$

**CONCLUSIONS**

In conclusion, buffalo milk exhibited large variability for both chemical composition and MCP. Significant relationships were found between production traits and coagulation properties. These results allowed a preliminary characterization of buffalo milk traits in northeast Italy. Further studies are needed to better investigate the variability of MCP and mainly the relationships between MCP and cheese yield.

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## Maternal origin of Medjimurje horse

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### ABSTRACT

*Maternal origin of highly endangered population of Medjimurje horse was analysed with intention to establish maternal lineages, to characterise its position among other horse breeds, to compare Croatian and Hungarian subpopulations and to compare Medjimurje horse with related breeds. To achieve those goals we sequenced fragment of mitochondrial control region (mtDNA D-loop) of 58 Medjimurje horses (27 from Croatian and 24 from Hungarian sub-population) and 7 Norikers from Austria (Carinthia region). We also analysed additional 95 sequences taken from the GeneBank database, representing large number of breeds and representative haplotypes, with particular stress on Arab Horse (17) and Renish German Draft Horse (24). The analyses have shown that out of 51 MH sequences there were 19 different maternal lineages (haplotypes). Out of 153 analysed sequences, nine haplotypes were unique for MH (six for MH-CRO and three for MH-HUN). It is interesting that among those unique haplotypes one was characteristic for nine MH-CRO horses and one for five MH-HUN horses. The phylogenetic analysis has illustrated that, although, maternal lineages of MH horse are present over distinct phylogenetic clusters there is some tendency of grouping around specific haplotypes and the similar pattern was noted between haplotypes of two MH subpopulations. Here, we have not found strong reasons why not to make common breeding program for MH. However, we should be aware that mtDNA analysis reflects remote origin and before proceeding with practical conservation decisions it would be necessary to analyse those two subpopulations based on microsatellite or SNP polymorphism.*

(Keywords: Medjimurje horse, haplotypes, phylogenetic analysis)

### INTRODUCTION

Medjimurje horse is autochthonous breed named after northern region of Croatia that is separated by two rivers, Mura and Drava. The history of this draft horse is linked to the Austro-Hungarian Empire where it was spread over the region that today is Austria, Croatia, Hungary and Slovenia. So, the breed is also recognized under names Muraközi ló (in hungarian language), Murinsulaner (in german language) or Medjimurski konj (in croatian language). The origin of the breed is linked to the Noric horse, Arab horse and cold blooded horses from Belgium, Croatia and Germany. Today, there is only 38 horses registered as purebred and the breed is critically endangered in Croatia, while found in traces in Hungary, Slovenia, and Poland. As a part of revitalization plan of Medjimurje horse, we have performed mtDNA analysis. The objective of this study was; a) to

establish maternal lines for future pedigree control and mating policy, b) to analyse maternal position of Medjimurje horse among world horses, c) to compare maternal origin of Medjimurje horse between Croatian (MH-CRO) and Hungarian (MH-HUN) sub-population, and d) to compare maternal origin of Medjimurje horse (MH) with Noriker (NO), Rhenish German Draught Horse (RD) and Arab horse (AR) as breeds that have influenced its formation.

## MATERIALS AND METHODS

Blood samples of 51 Medjimurje horses (MH) were collected from Medjimurje region in Croatia (27) and from breeding nucleus situated in Őrség National Park in Hungary (24) while blood samples from seven Norikers were collected in Carinthia region in Austria. All DNA was isolated using Sigma blood kit (Sigma-Aldrich, Germany) according to manufacturer's recommendations. Sequencing was done following the procedure described in *Aberle et al.* (2007). Thus, a 1260-bp fragment of the horse D-loop mtDNA, including parts of the tRNA-Thr, tRNA-Pro and tRNAPhe regions between nucleotides 15402 and 22, was amplified using the forward (5'-AACGTTCCCAAGGACT-3') and reverse (5'-GCATTTTCAGTGCCTGCTT-3') (Invitrogen) primers. The polymerase chain reaction (PCR) protocol was performed in a 20  $\mu$ l reaction mix containing approximately 50 ng of total DNA, 0.2  $\mu$ M of each forward and reverse primer and Master mix (Qiagen). The PCR was carried out in a iCycler (Biorad, USA) thermocycler and consisted of: an initial denaturation step at 95 °C for 15 min followed by 34 cycles at 94 °C, for 45 s, annealing at 62 °C for 45 s, and elongation at 72 °C for 80 s with a final elongation step of 10 min at 72 °C. PCR products were purified using ExoSAPIT (USB, Cleveland, OH) following the manufacturer's recommendations. DNA sequencing was preformed from the PCR product on an ABI 3130 DNA automated sequencer (Applied Biosystems, USA) using the ABI Prism Big Dye Terminator 3.1 Sequencing Kit (Applied Biosystems, USA). Obtained mtDNA sequences were aligned using the program CLUSTAL as implemented in the software MEGA 4.1 Beta 3 (*Tamura et al.*, 2007; available at <http://www.megasoftware.net/mega41.html>). Aligned sequences were truncated to the length of 276 bp (nucleotide positions from 15469 to 15745 according to *Xu and Arnason*, 1994; accession number X79547) to enable comparison with systematically known haplotypes and well known horse breeds (*Jansen et al.*, 2002).

All performed analyses were, further, based on the 276 bp truncated fragment. DnaSP v5.10 (*Librado and Rozas*, 2009; available at <http://www.ub.edu/dnasp/>) was used to estimate measures of DNA sequence variation within and between populations. The construction of the neighbour-joining tree was done using Kimura two-parameter model with complete deletion option (*Kimura*, 1980) by means of a MEGA 4.1 Beta 3 software. The tree was based on 58 target sequences (MH and NO) and 95 sequences additional 95 sequences taken from the GeneBank database, representing large number of breeds and representative haplotypes, with particular stress on Arab Horse (17) and Rhenish German Draft Horse (24).

## RESULTS AND DISCUSSION

In the 276-bp mtDNA D-loop fragment of 51 analysed MH sequences there were 26 polymorphic sites (9.4% for the total DNA sequence analysed), representing a total of 19 different haplotypes i.e. maternal lineages from the horse breeding approach, among which nine and seven were present only within MH-CRO and MH-HUN sub-

populations, respectively, while three haplotypes were present in, both, sub-populations (*Table 1*). Out of 153 analysed sequences, nine haplotypes were unique for MH (six for MH-CRO and three for MH-HUN). It is interesting that among those unique haplotypes one was characteristic for nine MH-CRO horses and one for five MH-HUN horses, see *Table 1*. The presence of a large number of horses within specific haplotypes is, most likely, a consequence of the high degree of relatedness caused by the small population size. From the NOK population, only three haplotypes were unique out of 153 sequences while each of seven analysed horses had different haplotype which is a consequence of the genetic population broadness of NO (*Table 1*). MH horses have shared haplotypes with RD (11), AR (5), NO (3) and Lipizzan (3) horses as well as with Icelandic (1) and Shetland (1) ponies and Kazah horse (1).

**Table 1**

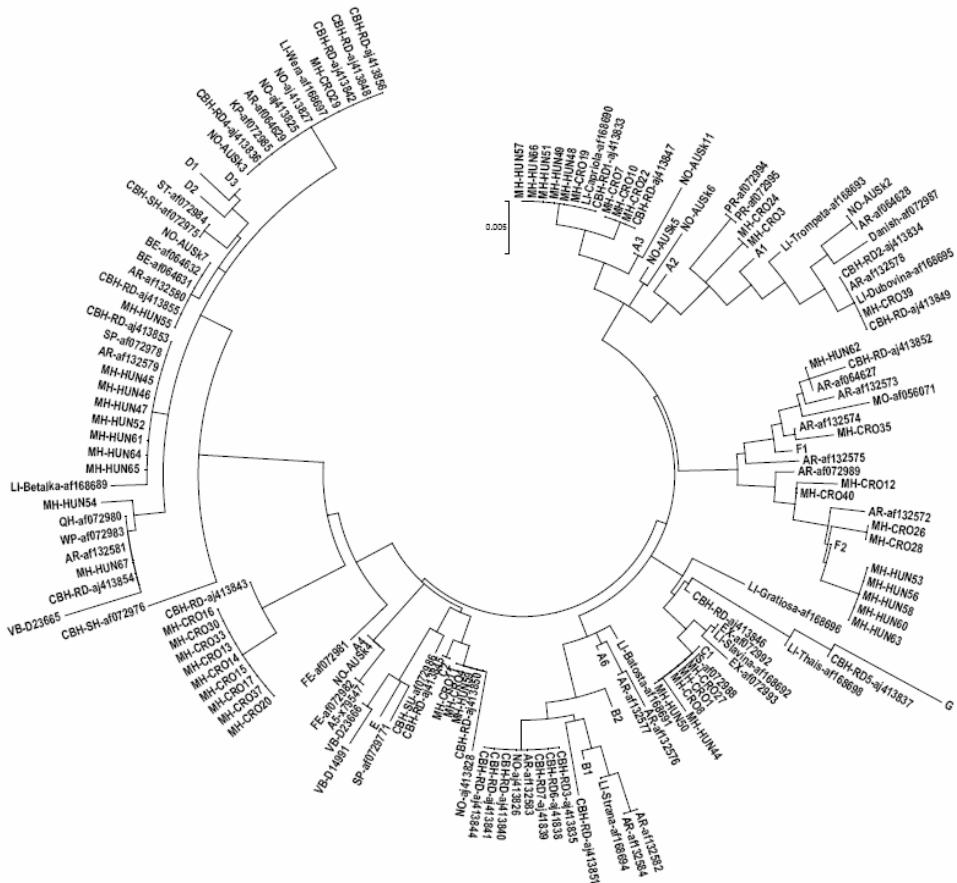
**Variable nucleotides in a fragment of DNA D-loop of the Medjimurje horse from Croatia (MH-CRO, 27 horses) and Hungary (MH-HUN, 24 horses) and Noriker horse from Austrian region Carinthia (NOK, 7 horses) haplotypes compared with reference sequence (Xu and Arnason, 1994; accession number X79547, in bold)**

15491	15495	15496	15497	15504	15527	15535	15541	15542	15543	15575	15587	15599	15603	15604	15605	15606	15637	15651	15652	15661	15668	15669	15674	15707	15713	15724	15730	15740	15742	15744	15745	MH-CRO	MH-HUN	NOK
C	T	T	A	T	T	C	A	C	C	-	G	A	T	T	C	T	G	C	A	A	T	G	A	G	T	C	G	G	-	T	A	A		
.	.	C	.	.	.	.	.	.	.	T	.	A	.	.	.	A	.	.	A	.	A	.	A	.	A	.	-	.	.	0	0	<b>1</b>		
.	C	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	-	.	.	.	0	0	1			
.	C	C	G	.	T	.	.	.	-	A	.	.	C	.	G	.	.	.	.	.	.	A	.	-	.	.	.	1	0	1				
.	C	.	.	.	.	.	.	T	-	A	G	T	.	T	.	G	.	A	.	C	.	C	.	-	.	.	0	0	1					
T	C	.	A	.	.	.	A	.	.	.	.	.	.	.	.	.	A	.	.	A	.	A	.	A	.	-	.	.	0	0	<b>1</b>			
.	C	C	G	.	T	.	.	.	-	.	.	T	C	.	G	.	.	.	.	.	T	A	.	-	.	.	.	0	1	1				
.	C	.	.	.	.	.	.	-	.	.	.	A	.	.	A	.	.	A	.	C	T	A	.	-	.	.	0	0	<b>1</b>					
.	C	.	.	.	.	.	-	.	.	.	.	G	.	A	.	.	A	.	A	.	A	.	-	.	.	3	0	0						
.	C	.	.	.	.	.	-	A	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	2	1	0					
.	C	.	.	.	.	.	-	.	.	T	.	A	.	.	.	.	C	.	A	.	-	.	.	.	1	0	0							
.	C	.	C	.	G	.	-	A	.	T	.	.	G	.	.	A	.	.	A	.	-	.	.	2	0	0								
.	C	.	.	.	.	-	T	-	A	G	T	.	.	G	.	A	.	.	A	.	-	C	.	2	0	0								
.	C	.	.	.	.	-	.	.	T	.	A	.	.	C	.	.	C	.	A	.	-	.	.	2	0	0								
.	C	.	.	.	.	-	.	.	T	.	A	.	.	C	.	.	C	.	A	.	-	.	.	G	3	1	0							
.	C	.	.	.	.	-	A	.	.	.	.	G	.	A	.	.	A	.	A	.	-	.	.	1	5	0								
.	C	.	.	.	.	-	.	G	T	.	.	.	.	.	G	.	C	.	A	.	-	.	.	1	0	0								
.	C	.	.	.	.	T	-	G	T	.	T	.	G	.	A	.	C	T	A	.	-	.	.	1	0	0								
.	C	.	.	.	.	-	.	T	.	A	.	.	C	.	.	C	.	A	.	-	.	.	1	0	0									
.	C	.	.	.	T	.	-	.	T	.	.	C	.	.	C	.	A	.	-	.	.	0	<b>1</b>	0										
.	C	C	G	.	T	.	.	-	A	.	T	C	.	G	.	.	.	A	.	-	.	.	0	7	0									
.	C	.	.	.	.	-	.	.	T	.	A	.	.	.	A	C	T	A	.	-	.	.	0	<b>5</b>	0									
.	C	C	G	.	T	.	.	-	A	.	C	A	.	G	.	.	.	.	.	-	.	.	0	<b>1</b>	0									
.	C	.	.	.	T	.	.	-	G	T	A	.	.	.	G	.	C	.	A	.	-	.	.	0	1	0								
.	C	C	G	.	T	.	.	-	A	.	T	C	A	.	G	.	.	.	A	A	-	.	.	G	0	1	0							

Haplotype frequencies for MH and NOK are given in the last three columns. Bold underlined haplotypes denote breed unique haplotypes out of 153 analysed sequences.

**Figure 1**

Neighbour-joining tree derived using the Kimura 2-parameter method with complete deletion option (Kimura, 1980) for 153 horse sequences (nucleotide positions from 15469 to 15745 according to Xu and Arnason, 1994; accession number X79547) representing phylogenetic position of Medjimurje horse in relation to worldwide horse breeds (notation taken from Jansen *et al.*, 2002). Prefix CBH stands for Coldblooded horses with exception made for the Medjimurje and Noriker horse



The phylogenetic analysis has illustrated that maternal lineages of MH horse are present over distinct phylogenetic clusters i.e. the analysis have failed to show monophyletic groups. For example, MH sequences were appearing in clusters A2, A3, D, F, E and G while not in B and, with exception of one large MH-HUN “family” and one MH-CRO horse, not in C clusters (for more detailed description of mtDNA phylogenetic clusters see *Jansen et al.*, 2002). This effect was previously noted in mtDNA studies of a large number of horses (*Jansen et al.*, 2002; *Aberle et al.*, 2007, *Royo et al.*, 2005; *Cozzi et al.*, 2004; *Iwanczyk et al.*, 2006). The similar pattern of similarity and distinction was noted between haplotypes of two MH subpopulations. Here, we have not found strong reasons

why not to make common breeding program for MH-CRO and MH-HUN subpopulations, particularly, as the effective population size of both population is extremely small. However, we should be aware that mtDNA analysis reflects remote origin and before proceeding with practical conservation decisions it would be necessary to analyse those two subpopulations based on microsatellite or SNP polymorphism (Achmann *et al.*, 2004; Druml *et al.*, 2006). In addition, the comparison of MH with other horses present in the region as an Posavina horse, Croatian Colblooded horse, Traditional Arab Horse and Bosnian Mountain horse on both mtDNA and nuclear markers would be required and this is our future goal. Furthermore, the sequence analysed here was restricted to 276-bp mtDNA D-loop fragment and we are currently analysing if larger mtDNA fragment does provide differentiation between haplotypes “occupied” with larger number of horses.

## CONCLUSIONS

The number of distinctive haplotypes does offer possibilities for establishment of maternal lineages based on mtDNA analysis which might be useful in preserving genetic diversity through optimisation of specific maternal contribution. The phylogenetic analysis has illustrated that, although, maternal lineages of MH horse are present over distinct phylogenetic clusters there is some tendency of grouping around specific haplotypes and the similar pattern, was noted between haplotypes of two MH subpopulations. Overall, the results of this analysis indicated the presence of specific genetic characteristics (mtDNA haplotypes) that urge needs for improving breeding (preservation) of Medjimurje horse.

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## Effect of age and event on post exercise values of blood biochemical parameters in show jumping horses

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### ABSTRACT

*Fitness is a certain metabolic status – as a result of training – which makes the equine athletes capable of good results in sport. Assess the fitness status in field conditions is therefore very important. Rather limited information is available on low class show jumpers, therefore the aim of this trial was study the effect of age and event on the biochemical blood parameters. During the winter indoor competition tournament (from October to February) blood samples were collected right after the first class of the last day of the event from all together thirty show jumpers (five, six and seven years old horses, five randomly selected horse from each year category at the first and at the last competition of the tournament). From the blood samples taken from the jugularis vein the plasma were separated and the lactate, LDH, CK, AST, glucose, cholesterol, triglyceride, total bilirubin and cortisol level were measured for evaluating the training status of the horses. Five year old horses had significantly lower lactate level compared to six and seven year old horses. At the end of the tournament horses had lower glucose and higher cortisol level in the plasma right after show jumping class. It can be concluded, that show jumping competitions on up to 100 cm height and a minimum of 300 m/min average speed do not involve substantial anaerobic energy supply. The frequent stress situation can elevate the cortisol response given. Multivariate methods applied on several post exercise blood parameter can be useful to detect slight differences in fitness.*

(Keywords: show jumping, horse, training, fitness)

### INTRODUCTION

The condition as a result of training is very important for every athlete including equine athletes. Standard exercise tests provide the possibility of to run the horse under controlled conditions, however data collected from a treadmill test do not reflect the horse's response to a sport event. Horses are generally exercised on an open field or indoor, having exposed to numerous other factors such as the rider, other horses, weather, spectators, decorations, terrain, etc. (Serrano *et al.*, 2001). Therefore, testing the biochemical and physiological changes due to field training or competition is important. The literature of equine athletes are relative abundant on data about Thoroughbreds, endurance and eventing horses. The few field test with show jumpers deal with more experienced horses and 130–150 cm high classes (Art *et al.*, 1990a,b). No information was found in the literature about the post exercise biochemical values of young horses competing on lower levels. Therefore, the aim of this study was to examine the effect of age and event on post exercise blood biochemical and enzymatic activity parameters.

## MATERIALS AND METHODS

*Experimental animals:* During the winter period (from October to February) the Indoor Show Jumping Championship is organized in Hungary. One location of the tournament is the Pannon Equestrian Academy at the Kaposvár University. Fifteen horses were randomly selected in three age categories (five, six and seven years old, five animal/age group) at the first (October 2009) and at the last (February 2010) event of the tournament (30 horse all together). Gender was not considered in the selection of animals to be tested.

*Blood sampling:* On the last day of the events, immediately after the first course two times 4 ml blood sample was taken from the jugular vein into the sampling tubes containing NaF-oxalate and Na-heparine. The blood samples were stored on ice until we spanned them. The samples were spanned at 4000 rpm for 3 minutes. Plasma were pipetted to an eppendorf tube and stored at a temperature -18 °C until the analysis.

*Laboratory analysis:* From the blood plasma samples lactate, lactate dehydrogenase (LDH), creatine kinase (CK), aspartate amino transferase (AST), glucose, cholesterol, triglyceride, total-bilirubin and cortisol level were determined in the laboratory of the Kaposi Mór Teaching Hospital (Kaposvár, Hungary) using Roche Modular SWA (Hoffmann-La Roche Ltd.) measuring system.

*Statistical analysis:* The experimental data were evaluated by the SAS 9.2 (SAS Institute Inc., Cary, NC, USA) statistical software package with GLM procedure. Interaction of age and event effect was not significant in the case of any parameter; therefore it had been left out from the general model and results presented as pooled. In case of significant main effect, the differences between the group means were tested by Tukey-test. Discriminant analysis used to test the hypothesis that treatments can be separated based on the blood parameters.

## RESULTS AND DISCUSSION

Five-year old horses had significantly lower lactate level than the 6 or 7 years old horses (*Table 1*). The average levels of the young horses were within the usual range (ref. value 0.5–2.0 mmol/l) indicated for resting animals, while older horses developed a characteristic of post exercise level with the mean value of 3.5 mmol/l. *Art et al.* (1990a,b) measured post competition lactate level between 6–9 mmol/l for horses competing on 130–150 cm obstacles with above 350 m/min speed. *Davie and Evans* (2000) demonstrated that exercise over 800 m at speeds 780–960 m/min results in blood lactate concentrations in the range of 4–19 mmol/l measured right after the exercise. The maximum lactate concentration determined by *Munoz et al.* (2008) after exercise in draft horses were 13.7 mmol/l, in racing horses were 12.8 mmol/l and in the endurance horses it was 2.9 mmol/l. The high intensity training programme consisting of gallop or trot at high speed resulted in a blood lactate concentration of more than 7 mmol/l in less than 3 min (*Guy and Snow*, 1977). These results indicate that lower class show jumping (up to 120 cm height) is not a strenuous exercise to horses. At the outset of any exercise energy is provided by the ATP stores and the ATP-creatine phosphate pathway. However these energy supplies deplete rapidly (up to one minute) and as the exercise intensity increasing the lactic acid pathway take over until the aerobic pathways can provide the energy requirement (1–2 minute) (*Ellis and Hill*, 2005). *Lindner* (1997) suggested that there is always enough oxygen available for the aerobic pathways, even during intensive exercise.

More likely the low energy providing capacity over time of the aerobic pathways is the answer why lactic acid pathway is used. The results of older show jumping horses support that theory. The warm up period usually last for about 30 minutes and involves light work and than riding the course takes 60–90 seconds as intensive work. The different results of the 5 years old horses can be answered by the difficulty of challenge they are facing with: the maximum height of the obstacles is 100 cm and the average minimum speed is 300 m/min, while for the 6 and 7 years old horses it is 110 cm, 325 m/minute and 120 cm, 325 m/min, respectively. The oxygen consumption during exercise is known to be close correlation with the energy expenditure during work. *Eaton et al.* (1995) and *Pagan and Hintz* (1986) estimated the energy expenditure based on oxygen uptake. They estimated similar values at the speed of 300m/min but markedly different at 400 m/min. These results suggest that up to 100 cm class competitions the aerobic energy providing pathway can meet the energy need of the horse.

Lactate dehydrogenase catalyzes the interconversion of pyruvate and lactate with parallel interconversion of NADH and NAD<sup>+</sup>. It converts pyruvate, the final product of glycolysis to lactate when oxygen is absent or in short supply, and it performs the reverse reaction during the Cori cycle in the liver. At high concentrations of lactate, the enzyme exhibits feedback inhibition and the rate of conversion of pyruvate to lactate is decreased, thus lactate to pyruvate is preferred. In this respect the elevated level (reference value 74–206 U/l, *Lumsden et al.*, 1980) of LDH is the result of anaerobic energy supply, however the differences in lactate level of age groups did not reflect in mean LDH values. Interestingly, *Art et al.* (1990a,b) found lower LDH levels in spite of higher post exercise lactate values, however horses were competing on higher classes.

The elevated level of creatine kinase reflects the intensity of actual workload and it can be easily double as seen in endurance horses and in our trial (*Table 1*). Again, *Art et al.* (1990a,b) measured much lower post exercise CK levels in horses competing in higher classes. It has been documented, that overtraining is not resulted in elevated CK activity (*Harris et al.*, 1997; *Hamlin et al.*, 2002). Nevertheless *Pritchard et al.* (2009) determined a 210 U/l reference value for Lahore working horses (Pakistan). They stipulated that the high levels of CK in working horses may indicate a low-grade, chronic muscle damage rather than a short-term and reversible effect of overwork. This assumption was based on the fact that the reference population was working for short periods daily. Elevated AST and total bilirubin levels can indicate muscle damage due to muscle strain from use, training. Our results fall in the wide reference ranges set for both light and working horses (*Lumsden et al.*, 1980; *Pritchard et al.*, 2009), but *Art et al.* (1990a,b) measured noteworthy lower AST values in high class show jumpers. Nevertheless, *Harris et al.* (1990) found persistently elevated AST (above 300 U/l) in thoroughbred racehorses with good race results. *Art et al.* (1990b) concluded that a single blood sampling could lead to a misinterpretation of post exercise biochemical values due to high interindividual variations. This regards particularly to blood lactate, LDH and CK. This phenomenon implies that the ability to compare different research results based on these biochemical parameters are limited.

All horses had normal serum cholesterol level, however it is tended to be higher at the end of the season. It has been shown that cholesterol level in blood plasma increases as a result of exercise but not due to conditioning (*Hambleton et al.*, 1980). The low serum triglyceride levels indicate that fat metabolism was not affected by show jumping. Plasma adrenaline, noradrenaline, b-endorphin and cortisol concentrations were increased by exercise in cool dry conditions (cortisol: 90 ng/ml) and were further increased by the same exercise in hot humid (cortisol: 130 ng/ml) conditions (*Williams*

et al., 2002). The degree of increase in plasma cortisol concentration appears to better reflect duration of workload rather than work intensity. Maximum plasma concentration was observed about 30 min after the end of a high intensity exercise (Marc et al., 2000). It has been also revealed that discrimination based on cortisol net increase (due to exercise) between endurance and dressage plus jumping trained horses are possible. Covalesky et al. (1992) demonstrated that more experienced horses have lower cortisol concentrations than less experienced ones after riding the course. In our study we were not able to detect this difference. Most probably the reason is that even young (5 year old) horses were already accustomed to the show environment.

Time of measurement (effect of event) had significant effect only on the blood glucose and cortisol level. One of the primary function of cortisol is to increase blood sugar level for instance in case of physical exertion. That can be the reason why we found significantly higher value while blood sugar level was lower. Cortisol plays important role in adaptation to stress. As summarized by Coenen (2005) different metabolic stresses – like jumping – can double the level of plasma cortisol. The significantly higher level at the last competition of the tournament can be the result of higher metabolic stress. Most horses did compete every second week during the winter season which together with the frequent transportation can cause higher reaction to these stress. Blood lactate concentration tended to be higher at the last event. Art et al. (1990b) found no difference in post competition lactate level over five competitions. Therefore, the slight difference in our case maybe accounted to the individual variation, as not the same horses were tested at the last event.

**Table 1**

**Blood parameters of different aged show jumpers at the beginning and at the end of winter competition period (mean values)**

Blood parameter	Age (year)			Event		RMSE	P values (age)	P values (event)
	5 (n=10)	6 (n=10)	7 (n=10)	First (n=15)	Last (n=15)			
Lactate (mmol/l)	1.5 <sup>a</sup>	3.5 <sup>b</sup>	3.5 <sup>b</sup>	2.3	3.4	1.6	0.010	0.059
LDH (U/l)	539.7	589.4	572.5	577.9	556.5	115.0	0.623	0.614
CK (U/l)	241.5	243.8	211.8	231.5	233.3	39.5	0.151	0.902
AST (U/l)	281.5	317.7	275.5	283.3	299.8	58.7	0.239	0.449
Glucose (mmol/l)	3.9	4.6	4.4	4.7 <sup>a</sup>	3.8 <sup>b</sup>	0.9	0.171	0.007
Cholesterol (mmol/l)	2.3	2.3	2.4	2.3	2.5	0.3	0.607	0.098
Triglyceride (mmol/l)	0.36	0.30	0.42	0.37	0.35	0.12	0.100	0.651
Total bilirubin (μmol/l)	27.7	29.8	32.2	28.2	31.4	7.1	0.376	0.220
Cortisol (nmol/l)	197.7	199.6	170.8	167.0 <sup>a</sup>	211.7 <sup>b</sup>	42.2	0.252	0.007

<sup>a,b</sup> Means within a row lacking a common superscript differ significantly P<0.05; RMSE: route mean square error

Since few significant differences were found with analyses of variance, we hypothesized that treatment groups may be still can be distinguished based on all the traits measured. Therefore, discriminant analysis was applied on the data set. We are aware that in this study we had too few observations compared to the number of traits to make firm conclusions on the result of this statistical analysis. Moreover statistically proven outliers were not excluded, because those values were physiologically sound. Therefore, even significant results interpreted as a tendency.

Three data were misclassified from the event groups of data (*Table 2*), and Pillai' Trace statistics show weak discriminant function. None of the data were misclassified from age groups (*Table 3*) and statistics indicates a strong discriminating function. It is a general view that only post exercise plasma lactate level and heart rate are good indicators of fitness, and other biochemical and enzymatic activity analyses are of limited usefulness (Art *et al.*, 1990a, Art *et al.*, 1994; Couroucé, 1999). However, our results indicate that the difference in fitness and metabolic status based on various biochemical parameters of different aged show jumpers could be possible in spite of few significant differences.

**Table 2**

Classification results of sampling time groups

From event group	Into event group				Total		Pillai' Trace	
	1		2					
	count	%	count	%	count	%	value	P
1 <sup>a</sup>	14	93.33	1	6.67	15	100		
2 <sup>b</sup>	2	13.33	13	86.67	15	100		
Total	16	53.33	14	46.67	30	100	0.55	0.030

<sup>a</sup>first event; <sup>b</sup>ast event

**Table 3**

Classifications results of age groups

From age group	Into age group						Total		Pillai' Trace	
	5		6		7					
	count	%	count	%	count	%	count	%	value	P
5	10	100	0	0	0	0	10	100		
6	0	0	10	100	0	0	10	100		
7	0	0	0	0	10	100	10	100		
Total	10	33.33	10	33.33	10	33.33	30	100	0.93	0.040

## CONCLUSIONS

Show jumping competitions on up to 100 cm height and a minimum of 300 m/min average speed do not involve substantial anaerobic energy supply. The frequent stress situation can elevate the cortisol response given. Multivariate methods applied on several post exercise blood parameter can be useful to detect slight differences in fitness.

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## Fattening of Improved Jezersko-Solčava weaned lambs with different diets

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### **ABSTRACT**

*The study was performed in a stable of Educational and Research Animal Husbandry Centre in Logatec. Thirty-five weaned Improved Jezersko-Solčava lambs, 16 females and 19 males, were included in the experiment. At the beginning of the study, lambs were on average 129 days (4.3 months) old and weighted on average 25.6 kg. Lambs were divided into four dietary groups by eight to nine lambs per group. Group I was fed with hay I and second harvest in the ratio 40:60 and 650 g of concentrate per lamb. Group II was fed with hay I and second harvest in the ratio 40:60, without concentrate. Group III was fed with hay II and supplemented with 650 g of concentrate per lamb, and group IV was fed only with hay II. Mineral-vitamin mixture and water were available to lambs *ad libitum*. The experiment lasted 40 days. Lambs were weighted every 10 days. Hay, second harvest, and concentrate offered to lambs were weighted every day. Data were analysed using SAS software. The statistical model included group (diet), sex, and birth weight. The average daily gains were: 196 g for Group I, 130 g for Group II, 169 g for Group 3, and 50 g for Group 4. The difference in the average daily gain among four groups of the lambs was statistically significant ( $P < 0.001$ ). We can conclude that diet influenced the average daily gain of the weaned lambs.*

(Keywords: lambs, fattening, diet, daily gain)

### **INTRODUCTION**

Improved Jezersko-Solčava (JSR) sheep is a result of improving the autochthonous Jezersko-Solčava (JS) sheep with Romanov (Kompan *et al.*, 1996). The JSR sheep is rather fertile breed, suitable for an intensive rearing of slaughter lambs. It is the most spread and the most numerous sheep breed in Slovenia. Breed JSR is mainly bred in the North and North-Western part of Slovenia. The population of JSR sheep is estimated to around 60,000.

The breed is fertile all year round, but most of sheep lamb in spring. The main production system in JSR breed is that lambs graze with their mothers on the pasture till they reach 25 to 35 kg. Lambs are then slaughtered. Some breeders, who have lambings in autumn, rear suckling lambs in stable, together with their mothers. There is also a tendency for fattening lambs after weaning, but different technologies, especially in feeding intensity, among breeders exist. In winter time, when lambs are fattened in stable, the diet is based on hay and sometimes cereal or concentrate supplementation. When lambs are fattened on pasture, they are often not supplemented, but their diet is only pasture.

Regarding feeding intensity in fattening lambs after weaning, daily gain of body weight varies among breeders (technologies). Žgur *et al.* (2003) fattened weaned JSR lambs with hay and commercial concentrate *ad libitum*. Daily gain of the lambs from birth to slaughter at 29 or 43 kg of live weight was over 300 g/day, regardless of their

live weight at slaughtering. Cividini et al. (2007) reported about 204 g of daily gain in pure JS lambs after weaning, which were fed with hay and cereals *ad libitum* or with pasture.

Daily gain of the lambs mainly depends on protein and energy level in their diet. Bahmaei lambs, which were fed three different metabolizable energy (ME) level diets (9.83 to 10.32 Meal/kg of dry matter), grew from 197 to 218 g/day (Hosseini et al., 2008). Increasing ME level in lamb's diets thus resulted in increasing growth performance. Some researches have shown that increasing the level of protein in high grain diets increases performance of the sheep (Dabiri and Thorney, 2004; Haddad et al., 2001; Kaya et al., 2009; Suliman and Babiker, 2007). In these studies, the protein concentration in the diet was 100 to 200 g in a dry matter basis. On the other hand, Ruiz Nuno et al. (2009) found that protein level has a minor effect on lamb performance.

Objective of the present study was to evaluate the effect of the winter diet on growth rate of weaned JSR lambs and their daily gain of body weight in a stable.

## MATERIALS AND METHODS

The experiment was performed in December 2009 and January 2010 in a stable of Educational and Research Animal Husbandry Centre Logatec. Thirty-five weaned lambs of Improved Jezersko-Solčava (JSR) breed were included. Sixteen lambs were females and 19 were males. Lambs were divided into four groups regarding of the sex, so that approximately half of the females and half of the males were in the group. There were eight to nine lambs in each group. The initial average body weight of the lambs was 25.4 kg. They were on average 129 days (4.3 months) old.

Before the beginning of the experiment, the 10-day adaptation period was performed, when lambs were shared and treated against parasites. Lambs were also adapting to the diet and new environment in this period.

Every group of the lambs was fed with its own diet. The diet was calculated using the average live weight of the lambs and nutrient requirements for fattening lambs (NRC, 1985). For this purpose the chemical composition of hay and second harvest was determined (*Table 1*). Metabolizable energy (ME) in hay and second harvest was estimated using the equations 1 and 2, by Babnik et al. (2001). In the course of the experiment, the quantity of the hay and second harvest was increased according to the lambs' consumption, while the quantity of the concentrate stayed the same.

**Table 1**

### Chemical composition of hay, second harvest and concentrate

	Hay I	Hay II	Second harvest	Concentrate
Dry matter (DM, g/kg)	877.3	878.1	847.4	880.1
Crude proteins (CP, g/kg)	76.5	61.9	124.4	205.0
Crude fat (CFat, g/kg)	15.0	13.0	25.4	
Crude fiber (CF g/kg)	326.0	338.5	271.7	
Ash (g/kg)	43.7	95.3	83.3	
Nitrogen free abstract (g/kg)	518.8	491.3	495.2	
Metabolizable energy (MJ/kg SS)	8.36	8.19	9.09	11.36

Estimation of metabolizable energy (ME, MJ/kg DM)

Hay – first harvest (*Babnik et al.*, 2001):

$$ME (MJ/kg^{-1} DM) = 13.00 - 0.01415 \cdot CF + 0.02256 \cdot CFat - 0.00482 \cdot CP \quad (1)$$

Second harvest (*Babnik et al.*, 2001):

$$ME (MJ/kg^{-1} DM) = 11.73 - 0.01129 \cdot CF + 0.04526 \cdot CFat - 0.00577 \cdot CP \quad (2)$$

The diet for Group I consisted of hay I and second harvest in the ratio 40:60 and 650 g of concentrate per lamb (*Table 2*). For Group II, the diet contained hay I and second harvest in the ratio 40:60, without concentrate. The diet for Group III consisted of hay II and 650 g of concentrate per lamb, and the diet for Group IV contained only hay II, without concentrate. The concentrations of crude proteins (CP) and ME in the DM of the diets differed among groups. The diet for group I contained the highest CP and ME concentration in DM (157 g CP and 11.16 MJ ME). Group IV contained the lowest concentrates of CP (70 g/kg DM) and ME (9.31 MJ/kg DM). Concentrate was fed individually, while second harvest and hay were fed per group. Mineral-vitamin mixture and water were available to lambs all the time.

**Table 2**

**Daily composition of the diet offered to lambs**

	<b>Group I</b>	<b>Group II</b>	<b>Group III</b>	<b>Group IV</b>
Hay I (g/lamb)	500	700		
Hay II (g/lamb)			1400	1700
Second harvest (g/lamb)	700	1200		
Concentrate (g/lamb)	650		650	
DM (g/day)	1603	1627	1801	1493
CP (g/kg DM)	157	127	122	70
ME (MJ/kg DM)	11.16	10.33	10.49	9.31

The experiment lasted 40 days. Lambs were fed once a day, in the morning. Firstly, Group I and Group III got the adherent quantity of concentrate. Lambs from these two groups were blocked into hayrack to eat its own concentrate, and they were left out afterwards. The refused concentrate was then removed from the hayrack. Hay and second harvest were then weighted and put into hayrack, regarding to the group. Before feeding next morning, the leavings of hay and second harvest in hayracks were removed. Lambs were weighted every 10 days, prior to the feeding. Daily gain of live weight was calculated from live weights of the lambs.

Statistical analysis was done using statistical package *SAS/STAT* (2001). Fixed part of the model was developed using the GLM procedure, where also descriptive statistics was done. Statistical model included group (diet;  $G_i$ ) with four levels and sex ( $S_j$ ) with two levels. Birth weight ( $W_k$ ) was treated as covariate in a simple linear regression. Litter size, initial age at the experiment, and initial age at the experiment did not affected daily gain of the lambs, therefore, they were excluded from the model.

## **RESULTS AND DISCUSSION**

The effect of winter diet on the growth rate of weaned JSR lambs and their daily gain of body weight in a stable was studied. Groups I and II were fed with hay I and second harvest in the ratio 40:60. Group I was supplemented with 650 g of the concentrate per

lamb. Hay II was fed to groups III and IV, while group III was supplemented with 650 g of the concentrate per lamb.

At the beginning of the experiment, the average live weight of the lambs was between 24.8 kg in group I (*Table 3*) and 25.8 kg in group II. In the 40 day's trial, the increase of the live weight was the highest (7.6 kg) in group I, which was fed with the richest diet, hay I, second harvest, and concentrate. Group III, which was fed with hay II and supplemented with concentrate, grew up for 6.8 kg. For 5.3 kg of live weight grew up group II, which was fed with hay I and second harvest, without supplement. The lowest average increase of live weight had lambs in group IV (only 2 kg), what was expected, regarding that they were fed only with hay. At the end of the experiment, lambs were in the average 169 days (5.6 months) old and they weighted on average 30.8 kg.

**Table 3****Basic statistical parameters for live weight of the lambs according to the group (kg)**

<b>Group</b>	<b>Day of the trial</b>	<b>n</b>	<b>Average</b>	<b>SD</b>	<b>Min</b>	<b>Max</b>
I	1	9	24.8	3.8	19.8	31.0
	20	9	29.4	5.3	23.5	37.5
	40	9	32.4	6.1	25.3	41.2
II	1	9	25.8	3.1	21.7	31.0
	20	9	28.8	3.4	24.7	34.5
	40	9	31.1	3.6	26.7	37.1
III	1	8	25.5	3.1	19.1	28.7
	20	8	29.8	2.8	24.3	33.7
	40	8	32.0	3.1	25.4	36.1
IV	1	9	25.7	3.0	22.4	31.3
	20	9	27.2	2.9	22.8	31.4
	40	9	27.7	2.7	24.1	30.9

n: number of the lambs; SD: standard deviation

As expected, daily gain of the body weight was the highest in group I, which was fed with the richest diet (*Table 4*). The average daily gain of these lambs was almost 200 g/day. Similar daily gain was achieved in pure JS weaned lambs (*Cividini et al., 2007*), which were fattened in the stable with hay and cereals *ad libitum* or with pasture. The diet offered to the lambs in group I contained the highest protein and energy level (157 g CP/kg DM and 11.16 MJ ME/kg DM—*Table 2*). *Hosseini et al. (2008)* fed lambs with different energy levels of diet (9.83 MJ ME/kg, 10.37 MJ ME/kg and 10.32 MJ ME/kg), and they found that lambs fattened with the highest energy level in the diet had the highest average daily gain, over 200 g/day). When *Haddad et al. (2001)* offered to lambs different crude protein concentrations (100, 120, 140, 160, and 180 g/kg DM) with 10.70 MJ ME/kg DM, the average daily gain was the highest (287 g/day) in the group fed with 160 g CP/kg DM. This diet was similar to the diet of group I in our experiment where lambs had also the highest daily gain among all four groups. *Ruiz Nuño et al. (2009)* fattened lambs also with different CP concentrations in the diet (140, 160, and 180 g CP/kg DM and 11.76 MJ ME/kg DM), but the average daily gain of the lambs (over 270 g/day) was not affected by the level of crude proteins. Group III of our experiment, which was also supplemented with concentrate, had lower daily gain (169

g/day) than group I. This group was offered quite lower CP concentration (122 g CP/kg DM) and also lower ME concentration (10.49 MJ ME/kg DM) in comparison to the group I. The lowest average daily gain had lambs in group IV, fed only with hay II. The average daily gain of this group was only 50 g/day. The diet of this group was the poorest as in proteins (70 g CP/kg DM) as in energy (9.31 MJ ME/kg DM) in comparison to other groups in the experiment. Regarding the level of CP and ME in the diets for our lambs, their daily gain, and nutrient requirements for fattening lambs (NRC, 1985), groups II, III, and IV were deficient especially in CP.

**Table 4**

**Daily gain of the lambs' body weight (g/day) according to the group**

<b>Group</b>	<b>n</b>	<b>Average</b>	<b>SD</b>	<b>Min</b>	<b>Max</b>
I	9	196	72	108	303
II	9	130	27	83	159
III	8	169	39	105	218
IV	9	50	36	-9	110

The statistical analysis showed significant differences ( $P<0.01$ ; *Table 5*) in the average daily gain of body weight among observed four groups. The diet thus influenced the average daily gain of the weaned JSR lambs. The average daily gain of the observed lambs was affected also by sex and birth weight. Litter size, initial body weight at the experiment, and initial age at the experiment did not affect the average daily gain of the lambs. Therefore, they were excluded from the model.

**Table 5**

**F- and P-values for the effects of group (diet), sex, and birth weight**

	<b>Group (diet)</b>	<b>Sex</b>	<b>Birth weight</b>
F-value	17.17	15.86	7.22
P-value	<0.0001	0.0004	0.0118

## **CONCLUSIONS**

The four groups of weaned JSR lambs, fed with different diets, had different average daily gain of the body weight. Lambs from the group I, fed with the richest diet, had the highest average daily gain (almost 200 g/day) in the 40 day experiment. As expected, lambs from group IV grew the less (50 g/day), but they were fed only with hay without supplementation.

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## **POSTER SECTION**





## New estimation possibilities the actual quality and value of grassland in Hungary

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### **ABSTRACT**

*The future way of grassland management is greatly affected by the new functions of the grasslands relative to the environment. The role of raising nutrition will be expanded by the role of keeping the natural resources. In the D-e-Meter grassland module we start the evaluation with the DM yield of the characteristic grass. This starting point is modified with the factors proper to the area. The measured and the estimated DM yield were compared to each other at 5 grasslands at Bőszénfa in Hungary. An important part of the sustainable developing is to find the adaptation to the local area and the nature. This aspect could be found in the multifunctional European Agricultural model and in the rural development too. The evaluation of grasslands in Hungary is not solved, the details we have are disused, so it is hard to plan the yields of the grasslands, we can estimate them a posteriori. To evolve an up-to-date evaluating system we analysed grasslands at the University of Kaposvár Deer Farm at Bőszénfa to find answers to the followings. Geological analysis of the humus, the N, P, K levels and the pH of the grasslands. Describe the botanical composition by the Balázs-method. The quality and the nutrition value of the grasslands. The yields of the grasslands, by annual. Analyzing of the results by the D-e-Meter system, and as a feedback to check the accuracy of the method.*

(Keywords: D-e-Meter, grassland, evaluation, yields, nutritive content)

### **INTRODUCTION**

Nowadays both the arable and the pasture land usage is controlled and has close connection to the environmental friendly farming. An important part of the possibilities of farm management is to know the actual quality and value of the fields. In the past several scientist try to build up method for the estimation to the quality and value of grassland. So the arable land evaluation and the controlled farming are important issues (Várallyay, 2003).

The important role of plant production and grassland management in Hungary has brought up the need to develop an informative system of arable evaluation. This method, which had been developing from the 1800's, was known as „aranykorona” value and was used till not long ago, in the 1990' by the arable lands privatization.

On the end of the 19<sup>th</sup> century one of the first classification of grasslands based on the botanical composition when Schröter and Stebler preformed the phyto-sociology, which analyzes the correlation between the local area and the plants. The best-known botanist of this method was Braun-Blanquet (1954). Authors (Türen, Knapp, Klapp, Könekamp, Köhnlein, Knoll, Petersen, Ellenberg, Stählin, Bocker) tried to harmonize the pure grassland qualification to the practice (Petersen, 1955).

In Hungary Thaïsz started to examine the grasslands by the botanical composition, but the first modern phyto-sociological examination was made by Soó (1941, 1964,

1965) and his followers. Other scientists Máthé and his followers, Juhász and Prettenhoffer also turned their attention to the problem of the grassland evaluation (Máté, 2003). Balázs (1960) examined botanical and economical evaluation. His system is used nowadays, and it's called „Balázs quadrate method”.

In the 1990's Vinczeffy (1995) created a grassland evaluation system, which characterizes the grasslands with three numbers. The problem with this method is that it doesn't deal with the geological and climate factors. So nowadays the correct evaluation of the grasslands is not solved, it is very difficult to plan the yields of the grasslands, the estimation is based on the routine.

An important part of sustainable development is to adapt to the local area and the nature. This aspect could be found in the multifunctional European Agricultural model and in the rural development as well. The “aranykorona” evaluation system is an obstacle for sustainability. Nowadays the evaluation of grasslands in Hungary is not solved, the data we have are dated, so it is hard to plan the yields of the grasslands. We have more than 1 million hectares of grasslands (more than 17% of the total agricultural area), so it is a very important question to find a well developed system to the estimation of the quality and value of grasslands.

During the last five years a new method was carried out in Hungary which hopefully will be suitable for the estimation of characteristics both the arable land and the grasslands. The name of the system is D-e-Meter method.

While the elaborated D-e-Meter method hopefully will be suitable for the arable farming, we have to aim to elaborate an up-to date method for the grasslands including the usable parts of the arable farming classification, and of course the special characteristics of the grasslands.

Classification of the grasslands can be solved in some ways. The most important from these are the followings: Baskay-Tóth (1966) separated the grasslands into 3 group according to the use: pasture, meadow and combined grasslands. Horn and Stefler (1990) classified the grasslands by the intensity of the usage of the grasslands separated them into three group (intensive, semi-intensive and extensive pasture). A new way of grassland classification was created by Dér et al. (2003), which was separated by the way of usage and the type of it (*Table 1*).

**Table 1**

**Grassland classification by the type of the grassland usage**

<b>Designation of the grassland</b>	<b>Categories</b>	<b>Size 1.060 e ha</b>	<b>Productivity t/ha DM</b>
Productive grassland	Unfertilized, or barely fertilized grasslands with medium productivity	54%	3–7
	Frequently fertilized grasslands with high productivity	3%	8–14
Area of outstanding natural beauty	Strictly sheltered grasslands	3%	No dates
	Non strictly sheltered, and other grasslands of outstanding area	15%	2–4
	Soil protecting grasslands	25%	1–2

The “Zöldmező” (Greenfield) society started the grassland evaluation at Keszthely in 1933. Later other county's grasslands were evaluated as well. The dates – after the

processing – were sent to the authority. During the 2<sup>nd</sup> World War the archives were lost, and after the end of the war the archives of the “Zöldmező” society were annihilated. After these happenings grasslands phyto-sociological composition was examined again. Hungary's arable land estate's potential fertility is high. The value of the arable lands is highly specified by the ecological factors. The bases of the productivity factors are the arable lands, so they must be in accord to each other. So man has to use very carefully the quantity, the composition, and the level of the resources.

The future way of grassland management is greatly affected by the new functions of the grasslands relative to the environment. This means that the role of raising nutrition will be expand by the role of keeping the natural resources.

As in other countries, the motivation to characterizing the arable lands was the increase of the taxes. By the end of the 18<sup>th</sup> century. II. Joseph enacted the cadastral survey of the arable lands. The works started in 1786, but the nobility balked the plan. After the fall of the 1848–49 revolution the taxes were assessed by the cadastre, which was the base of the arable land taxation till 1884. This base was measured in “korona” unit, which was the currency that time, but when the “korona” was not longer valuable, the unit was the “búza egyenérték” (wheat equivalence) which means that the value of it was equalized to the wheat's value. From 1924 we use “Aranykorona” unit.

When we say arable land evaluating, we often think about their classification by the productivity. In arable land evaluating people can use the income's capitalization methods as well. The market insists on the authentic and accurate qualifying of the arable lands. That is why the cadastre has to show the real quality number and fertility number of the arable land.

To evolve an up-to-date evaluating system we analyzed grasslands at the University of Kaposvár Deer Farm at Bőszénfa to find answers to the followings:

- pedologycal analysis of the humus, the N, P, K levels and the pH of the grasslands,
- describe the botanical composition by the Balázs-method,
- the quality and the nutrition value of the grasslands,
- the yields of the grasslands, by annual,
- analyzing of the results by the D-e-Meter system.

## MATERIALS AND METHODS

The experiments were carried out from autumn of 2005 (by soil analyzing) till autumn of 2008 at the Deer Farm of the Kaposvár University, at Bőszénfa. The five grasslands were the followings: Baltacim (6 ha), Egyenestető (23 ha), Kuti III. (9 ha), Pacsirta (19 ha), Templom Dél (20 ha). The meteorological details were measured by the Hungarian Meteorological Service in the near of Kaposvár.

We measured three samples per year from 2006 to 2008. The dates (yyyy.mm.dd.) of the cuts were the following (*Table 2*).

We cut samples from the grasslands in four repetitions by a 50×50 cm frame. The samples were analyzed at the Kaposvár University, Department of Chemistry and Biochemistry. They measured the weight of the samples, the DM contents, and quantified the crude protein, crude fat, crude fiber, crude ash and N-free extract by Weendei-analysis.

The statistical analysis was done with SPSS for Windows software at 5% significance level ( $P \leq 0.05$ ) by one-way anova. At the analysis of the results of the annual nutritive contents we used the weighted means of them. The weights were the DM yields of the three samples.

### Evaluation system of the D-e-Meter grassland module

In the following part of our publication we will introduce the basic theoretical aims of the D-e-Meter method and the possibility of its utilization in the practice. At the grassland point calculation method of the D-e-Meter system is based on the general yield ability of the field and we use some quality and quantity factors which has affect to the yield and stocking capacity of the grasslands during the growing period which are the followings (*Table 3*).

**Table 2**

#### Time of sampling (date)

First growth (1)	Second growth (2)	Third growth (3)
2006.05.15–25.	2006.07.5–15.	2006.09.15–25.
2007.05.05–15.	2007.07.15–25.	2007.09.20–30.
2008.05.10–20.	2008.07.10–20.	2008.09.25–10.05.

**Table 3**

#### D-e-Meter method for grasslands

<b>Starting point: Potential Dry Matter yield (t/ha)</b> In Hungary as the result of the ecological factors there are several types of grasslands. Very often we don't have any actual measured dates concerning the yield ability of the field therefore in our system we use dates from the literature of the most common 36 type of grasslands ( <i>Vinczeffy, 1995</i> ). We can use the measured dates of course, if we have more than five year's dates. This starting point could be modified with the qualifying factors of the field as the following:	
<b>Accumulation of water</b> Grasses are one of the cultures which need a lot of water. Grass growing is frequently held up by the low level of rainfall and the frequency distribution of it. The roots of the grasses work down till 10–15 cm deep, so if the weather is droughty, the only help for the grasses can be the depot in this deep. In our system we created five factors for the level of the ground-water in nine categories of the soil quality.	0.5–1.0
<b>Agro-ecological districts</b> The 7 main agro-ecological districts and its 35 subdistricts of Hungary – which have great affects to the agricultural production – The factors of them are between 0.6 and 1.6 in the D-e-Meter system.	0.6–1.6
<b>Gradient category</b> At the grasslands of slopes by increasing the gradient the effect of the rainfall become smaller and the caution of the sheet erosion is higher.	0.6–1.6
<b>Date of the sward establishment</b> The establishment – the soil management and the fertilization before it, and the composition of the grasses – has great effect to the productivity of the grassland. In the year of the establishment the factor is 0.8 because the cover of the grassland is not 100% yet. After the first year we calculate the factors from 1.2–1.6.	In the first year 0.8 After the first year 1.2–1.6
<b>Weathering of the year</b> The categories are almost the same as in the arable farming method in the D-e-Meter system, but we classified five group, and the factors are between 0.8–1.2.	0.8–1.2

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<b>Intensity of cultivation</b> The categories in this section are the followings: intensive, semi-intensive and extensive. Intensive grasslands are well fertilized and used (grazing or cutting) frequently. Extensive grasslands are unfertilized and rarely used.	0.7–1.5
<b>Quality factor</b> Grasslands are a complex company of annual species like grasses and legumes and other plants. The nutritive values of them are different. The yields of the different type of grasses could be modified with the quality factors, and could give a more accurate figure to the real value of the grassland.	0.85–1.0

## RESULTS AND DISCUSSION

By evaluating the results of the researches we used the botanical and the geological records to analyze the botanical and the geological records to analyze the fresh mass, the dry matter yield and the crude protein yield sorted by the areas. The yields of the grasslands were compared to each other by the fresh mass (t/ha), the DM yield (t/ha) and the crude protein yield (kg/ha) in the examination years (*Table 4*).

**Table 4**

### Yields of the grasslands

Area	Year	Fresh mass (t/ha)	DM yield (t/ha)	Crude protein yield (kg/ha)
Baltacim	2006	16.5 <sup>a</sup>	5.4 <sup>a</sup>	631 <sup>a</sup>
	2007	14.3 <sup>ab</sup>	5.6 <sup>a</sup>	568 <sup>a</sup>
	2008	13.8 <sup>b</sup>	5.6 <sup>a</sup>	543 <sup>a</sup>
Egyenestető	2006	20.2 <sup>a</sup>	5.8 <sup>a</sup>	630 <sup>a</sup>
	2007	14.3 <sup>b</sup>	4.8 <sup>b</sup>	499 <sup>b</sup>
	2008	23.2 <sup>c</sup>	7.7 <sup>c</sup>	828 <sup>c</sup>
Kuti III.	2006	13.0 <sup>a</sup>	3.9 <sup>a</sup>	400 <sup>a</sup>
	2007	12.1 <sup>a</sup>	4.0 <sup>a</sup>	437 <sup>a</sup>
	2008	11.7 <sup>a</sup>	3.8 <sup>a</sup>	493 <sup>a</sup>
Pacsirta	2006	22.7 <sup>a</sup>	6.1 <sup>a</sup>	825 <sup>a</sup>
	2007	8.2 <sup>b</sup>	2.7 <sup>b</sup>	398 <sup>b</sup>
	2008	23.1 <sup>a</sup>	7.0 <sup>c</sup>	808 <sup>a</sup>
Templom Dél	2006	23.6 <sup>a</sup>	7.4 <sup>a</sup>	993 <sup>a</sup>
	2007	15.1 <sup>b</sup>	5.5 <sup>b</sup>	604 <sup>b</sup>
	2008	17.6 <sup>c</sup>	7.6 <sup>a</sup>	713 <sup>b</sup>

<sup>a,b,c</sup> P<0.05

At Baltacim we couldn't find significant difference between 2006 and 2007 at the fresh mass product per hectare. The DM yield per hectare and the crude protein yield per hectare in the experimental years doesn't show significant difference. The drought of year 2007 had great effect to the arable land production, but in grassland production only in the 2<sup>nd</sup> cut, so the other two cuts could equalize the yields.

At Kuti III. we couldn't find any significant differences among the experimented yields. At Pacsirta the annual change of the weather has greater effect to the yields. In

2007 the unsuitable management had an effect too, so the differences were significant in all the measured parameters.

At Templom Dél the differences were significant in the fresh mass yield in the experimental years. The level of the yields were influenced by the weather and the land use management.

#### **The D-e-Meter evaluation associates to the measured yields**

At the examined areas we estimated the yields by the D-e-Meter system grassland method in the experimental years. The estimated and the measured DM yields were from the three cuts of the grasslands, and are showed on *Table 5*. The statistical evaluation was made by one sample T-test to compare the estimated and the measured DM yields.

**Table 5**

**The D-e-Meter grassland evaluation associates the measured yields at Bőszénfa in 2006-2008**

<b>Area</b>	<b>Year</b>	<b>Estimated DM Yield t/ha</b>	<b>Measured DM Yield</b>
Baltacim	2006	5.4 <sup>a</sup>	5.4 <sup>a</sup>
	2007	4.8 <sup>a</sup>	5.6 <sup>b</sup>
	2008	4.8 <sup>a</sup>	5.6 <sup>a</sup>
Egyenestető	2006	6.7 <sup>a</sup>	5.8 <sup>b</sup>
	2007	6.0 <sup>a</sup>	4.8 <sup>b</sup>
	2008	7.5 <sup>a</sup>	7.7 <sup>a</sup>
Kuti III.	2006	4.5 <sup>a</sup>	3.9 <sup>b</sup>
	2007	3.0 <sup>a</sup>	4.0 <sup>b</sup>
	2008	4.5 <sup>a</sup>	3.8 <sup>b</sup>
Pacsirta	2006	6.7 <sup>a</sup>	6.1 <sup>a</sup>
	2007	2.0 <sup>a</sup>	2.6 <sup>b</sup>
	2008	6.7 <sup>a</sup>	7.0 <sup>b</sup>
Templom Dél	2006	7.1 <sup>a</sup>	7.4 <sup>a</sup>
	2007	4.8 <sup>a</sup>	5.5 <sup>b</sup>
	2008	5.4 <sup>a</sup>	7.6 <sup>b</sup>

<sup>a,b</sup>P<0.05

Although there are significant differences between the estimated and the measured DM yields, the values show that the D-e-Meter method could give a good basic to plan the management. By improving this method with 0.2–0.4 t/hectare we can give estimation for the DM yield without significant differences.

#### **CONCLUSIONS**

We can draw the following conclusions from the experiments:

- Drought has the greatest effect to the botanical content, the cover of the plants and the DM content.
- In dry years the total number of the species can dramatically decrease, so this creates empty areas, and the number pioneer species (in the first place weeds) and the mass of them increases.

- The DM content of the grasslands increased caused by the drought year of 2007, but the crude protein per DM kilograms and the crude fiber per DM kilograms were almost the same. The yields of the grasslands are influenced to greater extent by the weather of the year (especially the rainfall) than the nutritive contents.
- By evaluating the yields we can say that grasslands with greater yields have greater effect by the changes of the weather than by the management.
- Grasslands can compensate the environmental effects, which have impact on one mow, compared to the field cropping species.
- The deviation between the estimated and measured DM yields is 0.05–1.2 t/ha, which shows high level of deviation, but greater margin of error is in the droughty year, so we have to correct this point of the method.
- Of course we must correct the accuracy of the method by further dates of the production, but in all the results certify the conduciveness of the D-e-Meter system in the near future.

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## Deep-litter pig keeping (A review)

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### **ABSTRACT**

*The study objective was to elaborate the system of pig keeping on deep litter, pointing out advantages and disadvantages of such system in comparison with conventional pig breeding systems. The study also presents future perspectives of deep litter pig keeping in Croatia. Raising of pigs on deep litter has only recently become acceptable as a way of production of high quality pigs and pig meat, mostly because of its ecological aspect and beneficial effects on pig welfare and health. Some contradictory research results referring to production and slaughtering characteristics, as well as to microclimatic conditions, indicate that further efforts have to be made in order to optimize deep litter pig keeping. Interventions in zootechnical procedures are aimed at optimization of pig production on deep litter in suitable climatic environment.*

(Keywords: pigs, deep litter, welfare, health, meat quality)

### **INTRODUCTION**

Regardless of keeping system, production of pigs should be oriented at provision of good production outcomes at the lowest possible costs. Such production results can be achieved only if considering all factors that affect production efficiency, as efficiency and profitability of overall pig production depend on the achieved production results. There is currently an emphasis put on welfare and health of pigs, as well as on ecological production of pig meat, which contributes to development of alternative pig keeping systems. Conventional ways of pig production cannot fulfill the requirements with respect to pig welfare and health. Recent trends indicate increasing popularity of deep litter pig keeping in countries with developed pig production, as well as in Croatia. Compared to conventional pig keeping systems, raising of pigs on deep litter has many advantages, such as beneficial effects on pig health and welfare, reduced occurrence of injuries and diseases, lowered production costs and ecological aspect (Margeta *et al.*, 2004; Kralik *et al.*, 2005). Referring to production and slaughtering characteristics, there are contradictory research results available, which point out the need for further researches on this topic in order to find optimal zootechnical and zoohygienic solutions necessary for adjusting deep litter pig keeping to specific conditions of a particular area. The aim of this review was to elaborate deep litter pig keeping system, pointing out advantages and disadvantages of such system compared to conventional pig farming systems and presenting possibilities of wider implementation of such system in Croatia.

### **Production characteristics of pigs**

There are some contradictory results referring to reproductive and production characteristics of pigs kept on deep litter. Gutzmirtl (2009) stated that sows on deep litter had identical

number of piglets as sows kept on cross-bared floor, but the level of stress was significantly lower for sows on deep litter. Weaned pigs kept on deep litter weighed more and were more vital and healthy than weaned pigs kept conventionally. *Klont et al.* (2001), and *Honeyman and Harmon* (2003) stated that pigs kept on deep litter had in the finishing phase of fattening higher feed consumption, less feed conversion per kg of weight gain and higher average daily gain. *Beattie et al.* (2000) and *Kralik et al.* (2004) pointed out that pigs on deep litter had more favorable consumption and feed conversion than pigs kept in conventional systems. *Lambooij et al.* (2004) stated that pigs kept on deep litter had significantly higher weight of warm carcasses and better water holding capacity than pigs kept on cross-bared floor. Better capacity of water holding in muscle tissue was explained by the above mentioned authors as a consequence of pigs' intensive movement during fattening, which affected firmness and better structure of muscular tissue. *Margeta and Kralik* (2006) determined better slaughtering characteristics of pigs kept on deep litter (*Table 1*). *Gentry et al.* (2002) and *Maw et al.* (2001) did not detect significant differences in production characteristics between pigs kept on deep litter and conventionally fattened. On the other hand, *Gentry et al.* (2002), and *Morrison et al.* (2003) determined increased deposition of fatty tissue in carcasses of pigs kept on deep litter, as well as worse feed conversion and growth characteristics. *Kralik et al.* (2005) stated that keeping of pigs on deep litter had unfavorable effect on daily weight gain. The authors of the present study explain such occurrence by the fact that pigs on deep litter had less frequent feeding, but their average feeding lasted longer than in pigs kept within conventional systems. Less frequent feeding of pigs bred conventionally, as well as longer interval of feed consumption affected tissue division in pigs' body to be more in favor of fatty tissue than of muscle tissue.

**Table 1****Slaughtering traits of fattening pigs kept on deep litter and conventionally**

Trait	Statistical indicators	Way of fattening	
		Deep litter system	Conventional system
Live weight before slaughter (kg)	$\bar{x}$	100.70 <sup>b</sup>	109.13 <sup>a</sup>
	sd	2.70	4.92
	CV	2.68	4.51
	$s \bar{x}$	0.54	1.00
Fat thickness - S (mm)	$\bar{x}$	12.12 <sup>a</sup>	14.13 <sup>a</sup>
	sd	7.20	6.69
	CV	59.42	47.35
	$s \bar{x}$	1.44	1.37
Muscle thickness - M (mm)	$\bar{x}$	68.52 <sup>b</sup>	70.04 <sup>ab</sup>
	sd	6.00	4.89
	CV	8.76	6.99
	$s \bar{x}$	1.20	1.00
Lean meat percentage (%)	$\bar{x}$	59.29 <sup>a</sup>	57.71 <sup>a</sup>
	sd	6.42	6.63
	CV	10.82	11.48
	$s \bar{x}$	1.28	1.35

Source: *Margeta and Kralik*, 2006.

a,b P&lt;0.05

*Klont et al.* (2001) concluded that keeping of pigs on deep litter had many benefits on production and slaughtering characteristics of pigs. Pigs on deep litter were able to move around freely, which reduced stress susceptibility and increased growth and meat quality.

### Microclimatic conditions

Deep litter as a means of pig keeping absorbs gases and moisture from excrement and urine, and in this way it directly provides for better microclimatic and zoohygienic conditions within facilities. Almost every material available in larger amounts that has certain absorption abilities and is not damaging for pigs' health and production characteristics, can be used as deep litter. There are also opposite research results with respect to microclimatic conditions of facilities. *Myczko* (2002) determined that emission of ammonia was significantly lower in facilities with deep litter than in conventional facilities, and *Klemola* (1998) stated that concentration of dust in the air was significantly lower in facilities with deep litter than in conventional ones. However, *Jeppsson* (2002) pointed out that pigs kept on deep litter had increased body temperature due to their physical activity, which consequently increased the content of  $\text{NH}_3$  and  $\text{CO}_2$ , as well as relative air moisture in the facilities with deep litter. *Margreta et al.* (2004) did not determine statistically significant differences between facilities with deep litter and without it with respect to air temperature and humidity, air circulation and level of carbon dioxide ( $\text{CO}_2$ ) and ammonia ( $\text{NH}_3$ ) in the air, but the values of carbon dioxide and ammonia were lower in deep-litter barns than their concentration in conventional systems (Table 2).

**Table 2**

#### Microclimatic parameters on deep-litter and conventionally keeping system

Parameter	Statistical indicators	Way of fattening	
		Deep litter system	Conventional system
Air humidity (°C)	$\bar{x}$	67.33 <sup>a</sup>	71.23 <sup>a</sup>
	sd	6.23	4.49
	CV	81.41	94.32
	$s \bar{x}$	1.69	1.61
Air circulation speed - (m/s)	$\bar{x}$	0.23 <sup>a</sup>	0.22 <sup>a</sup>
	sd	0.04	0.05
	CV	97.41	83.19
	$s \bar{x}$	0.01	0.02
$\text{NH}_3$ (ppm)	$\bar{x}$	9.36 <sup>a</sup>	11.39 <sup>a</sup>
	sd	1.71	1.69
	CV	74.26	75.36
	$s \bar{x}$	0.42	0.43
$\text{CO}_2$ (vol. %)	$\bar{x}$	0.12 <sup>a</sup>	0.14 <sup>a</sup>
	sd	0.04	0.06
	CV	71.67	64.15
	$s \bar{x}$	0.02	0.02

<sup>a</sup> P<0.05

## **Facilities**

Facilities used for production of pigs on deep litter are still not commonly found in Croatia. This fact is quite surprising if considering low construction costs for such facilities and availability of litter which is produced on Croatian arable land in great amounts. Advantages of facilities for deep litter pig keeping are seen in up to 40% lower costs of construction if compared to conventional facilities, as they do not require expensive equipment, crossbars, channels, lagunas, etc. Facilities with deep litter require also more space per an animal, which positively affects animals' behavior (Sargent, 2001), reduces aggressiveness and injuries occurrence and enables better social and hierarchical relations within a group. Furthermore, facilities for keeping of pigs on deep litter do not need investments in ventilation systems, as air circulates naturally. All stated positive effects of deep litter keeping system on pigs' health and behavior have also a by-side effect on improvement of production and slaughtering characteristics of pigs.

## **CONCLUSIONS**

Keeping of pigs on deep litter is an alternative, ecological pig production system. In comparison with conventional keeping systems, it has many advantages, the most important of which are its positive effect on pig welfare and health, and reduced disease and injury occurrence, which is in line with the EU requirements of provision for animal welfare. Referring to slaughtering characteristics, there are some opposite results on the beneficial effects on deep litter pig keeping. Microclimatic environment in facilities with deep litter is better than in facilities for conventional pig production, if required microclimatic and environmental norms are followed. Construction of facilities for deep litter pig keeping is up to 40% cheaper than for conventional facilities, and there are no limitations referring to environment protection. Keeping of pigs on deep litter is a suitable way of pig production for family farms in Croatia.

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## Limits in extensive swine husbandry – a case study of Turopolje pig production system in Croatia

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### ABSTRACT

*Turopolje pig production system could be define as an extensive swine production system in which large areas of pasture and woodland are used (more than 3 ha/pig) with low investment in housing and feeding. The aim of this paper was to analyze the state in Turopolje pig population in relation to reproductive traits and brucellosis outbreaks. The obtained results showed the slow increase of breeding (12 vs. 138 sows and 3 vs. 18 boars) and effective population (9.6 vs. 63.7) from 1996 to 2009, respectively. As main reasons fore that state were detected low farrowing index (1.0 to 1.33), small litter size (4.44 and 4.21 total born and born alive piglets, respectively), high mortality of piglets in suckling period (up to 35%) and consequently low number of weaned piglets per sow per year (4.45) as well as brucellosis outbreaks (44.16 % and 30.12 % serologically positive blood samples in year 2008 and 2009, respectively). In order to changes of that state urgent development of health management program and improving in production system (housing, feed supplementation, keeping area) are needed.*

(Keywords: Turopolje pig, production system, litter size, brucellosis)

### INTRODUCTION

The extensive swine production could be defined as a system in which large areas of land are used with low investment in housing and feeding (Honeyman *et al.*, 2005). In this system pigs are mostly kept outdoor and have a freely access to pasture and soil. If this production system is coupled with intensive management practices can result in acceptable production level, high quality of pork and excellent animal welfare (Honeyman *et al.*, 2001). Generally is accepted that old pig breeds such as Turopolje pig is well adapted to extensive conditions (Dikić *et al.*, 2002). Turopolje pig is originally created as a pig adapted to extensive farming in periodically flooded forests of English oaks and marsh meadows in continental climate (Dikić *et al.*, 2002; Dikić *et al.*, 2006). In all phases of “Turopolje pig production system”, pigs were kept outdoor with extensive using of woodland (>3 ha/pig), except the farrowing and suckling period in which sows and pigs are kept in wooden field building type “stanci” with restricted moving area in wood environment. Feeding is based on utilization of natural resources such as pasture, acorn and soil with addition of 0.5 kg of corn seed/pig, especially in suckling and late fattening period as well in winter season with snow (Jurić *et al.*, 1997). Production level of this production system could be highly influenced by weather conditions and control of diseases. In the past it was reported about outbreaks of some

diseases (swine fever, brucellosis, leptospirosis) which limits the increase of breed size and production level (Cvetnić et al., 2002; Cvetnić et al., 2003; Roić et al., 2007). The purpose of this paper was to analyze the state in Turopolje pig population in the period from 1996 to 2009 in relation to reproductive traits and brucellosis outbreaks.

## MATERIALS AND METHODS

### The size of population

The size of breeding population (number of sows and boars, total reproducers) and number of farms were analyzed in period between 1996 and 2009 using a data of Annual reports - Pig breeding of Croatian Livestock Center (CLC, 1997 to 2010). The effective size of population (Ne) was calculated according to formula by Falconer and Mc Key (1996):

$$Ne = 4 \cdot \frac{Nm \cdot Nf}{Nm + Nf} \quad (1)$$

where Nm is number of male (boars) individuals and Nf is number of female (sows) individuals.

### Reproductive parameters

Data were collected in the period from 2000 to 2009, in several farms, representing two major parts of the current farming area of Turopolje pig, Turopolje field – woodland area near Zagreb and Natural park "Lonjsko Polje". It was collected data from 529 litters and 388 sows registered in Herdbook of Turopolje pig breed. Sows were kept on all farms under similar conditions in outdoor production system with extensive using of woodland and pasture. Collected data include: farrowing index, number of piglets (total born, born alive and weaned piglets) per litter and per sows and year.

### Serological test

During the year 2008 and 2009 blood samples from 385 and 83 animals were collected, respectively and were serologically tested to *Brucella suis* infection. The Complement Fixation Test (CFT, Institut Pourquier, Montpellier, France) was used as test for serological testing on brucellosis. The result of the test was interpreted according to producer recommendations.

## RESULTS AND DISCUSSION

The program of renewal and conservation of Turopolje pig breed was started in the year 1996. At the beginning in herdbook were registered only 12 sows and 3 boars. In the next three years (*Table 1*) population was standardized and number of breeding animals were consolidate to 36 sows and 6 boars in 1999, what could be used as a basic year for considerable increase in number of breeding population. In the period from 1999 to 2008 the size of breeding population was increased near the 5 times in number of sows and 6 times in number of boars registered in herdbook. The increase in number of boars and sows resulted in the increase size of effective population, from 20.6 to 98.6 in year 1999 and 2007, respectively. According to the FAO criteria for determining breeds at risk (Loftus and Scherf, 1993), Turopolje pig was in critical status from 1996 to 2003 and in the status of endangerment from 2003 to 2008. But in 2008 the numbers of sows and boars was decreased about 21% in relation to 2007 and was not significantly changed during the year 2009, primarily due to outbreak of brucellosis in Turopolje pig population.

**Table 1**

**Breeding population of Turopolje pig in Croatia**

Year	Total reproducers	Sows	Boars	Effective size of population	Farms
1996	15	12	3	9.6	-
1997	25	17	8	21.8	-
1998	19	13	6	16.4	-
1999	42	36	6	20.6	5
2000	45	40	5	18.8	5
2001	50	45	5	18.0	4
2002	74	70	4	15.1	3
2003	105	99	6	22.6	2
2004	125	116	9	33.4	2
2005	143	129	14	50.5	3
2006	150	137	13	47.5	4
2007	193	164	29	98.6	4
2008	145	130	15	53.8	5
2009	156	138	18	63.7	5

Source: Annual report – pig breeding, HPA (1997–2009).

As shown in *Table 2*, in the year 2008 and 2009 about 44% and 30% of tested blood samples of Turopolje pigs were serologically positive to *B. suis* infection, respectively. According to Croatian legislation, all serologically positive animals to *B. suis* infections could be excluded from population. In spite of this, a lot of animals were excluded from breeding population what could explain decrease in number of breeding animals in Herdbook in 2008 and similar number of breeding animals in 2009.

**Table 2**

**Seroprevalence of brucellosis in Turopolje pig breed in year 2008 and 2009.**

Year	Total number of blood samples	Serological positive blood samples	
		Total	%
2008	385	170	44.16
2009	83	25	30.12
Total	468	195	41.67

Swine brucellosis is contagious disease in pigs caused by the bacteria, *Brucella suis* that could be transmitted to humans. Infection occur primary in domestic and feral pigs, while wild boar (*Sus scrofa*) and/or European hare (*Lepus europeus*) could be assume as a natural reservoir of *B. suis*. The most common symptoms of brucellosis include non-specific reproductive disorders such as infertility, abortion at any stage of gestation, stillbirth, weak pigs and *orhitis* in boars. The disease spreads by semen during coitus and by ingestion or inhalation of bacteria in reproductive fluids, placenta, aborted fetuses, urine or milk. Pigs kept in extensive production systems such as Turopolje pig

production system are highly exposed to *B. suis* infection. In Turopolje pig production system pigs are mostly kept outdoors with extensive using of pasture and woodland. Housing is based on natural covering or in primitive woodenly buildings type "Stanci". Pigs often graze together with other species of domestic animals and mate naturally. All of this increases the possibility of direct contact among pigs from different owners as well as with wild boar (Cvetnic et al., 2003). In addition, earlier study (Cvetnic et al., 2009) suggest that brucellosis occur enzootic in Croatian population of wild pigs, especially in breeding area of Turopolje pig. In spite of this, presence of *B. suis* in pigs environment and extensive production system could be explaining for occurring and high prevalence of brucellosis in Turopolje pig population. It was reported about some other contagious diseases (leptospirosis, swine fever) in pigs and/or in wild animals in breeding area of Turopolje pig (Cvetnic et al., 2002; Roic et al., 2007), what emphasize problems in control of infectious diseases in Turopolje pig production system. In addition, slow increasing of breeding population could be explained due to low farrowing index, high mortality during suckling period, low interest of family farms and lack of pastures and forests areas for keeping of pigs (Table 3).

**Table 3****Reproduction traits of Turopolje pig breed**

Year	Number of litters	Farrowing index	Number of piglets					
			Average per sow			Average per litter		
			Total born	Born alive	Weaned	Total born	Born alive	Weaned
2000	37	1.12	6.24	6.15	4.85	5.57	5.49	4.32
2001	23	1.10	6.14	5.95	3.90	5.61	5.43	3.57
2002	11	1.00	-	-	4.55	-	-	4.09
2003	36	1.00	4.78	3.33	2.83	4.78	3.33	2.83
2004	120	2.00	6.57	6.57	1.32	3.29	3.28	0.66
2005	39	1.00	3.21	3.13	3.08	3.21	3.13	3.08
2006	20	1.33	7.40	7.40	7.13	5.55	5.55	5.35
2007	34	1.17	6.66	6.48	6.41	5.68	5.53	5.47
2008	29	1.16	5.96	5.88	5.32	5.14	5.07	4.59
2009	60	1.02	5.64	5.34	5.15	5.55	5.25	5.07
<b>Total</b>	<b>529</b>	<b>1.19</b>	<b>5.26</b>	<b>5.02</b>	<b>4.45</b>	<b>4.44</b>	<b>4.21</b>	<b>3.90</b>

The results in Table 3 shown low farrowing index (in average 1.19) and high variability in number of total born piglets per litter and sow (3.21 to 5.68 and 3.21 to 7.40 piglets per litter and sow, respectively.) In the same time more than 90% of total born piglets were born alive, while mortality during suckling period varied between 2% and 35%. Except was in year 2004 when was observed 2.0 litters per sow, but only 3.29 total born piglets/litter and 80% of mortality during suckling period. These results suggest that Turopolje pig production system as an extensive system is more dependent on environmental conditions (weather conditions, keeping area and availability of natural feed resources – growing plants, forest fruits, and soil animals) and one farrowing per year in warm season with abundance of vegetation is sufficient. Contrary, if used as a usually practice two farrowing per year one of these come in cold and humid season what could have a strong influence on survival of young piglets. The whole population

of Turopolje pig was fragmented in two large herds with more than 50 reproducers each and several small family herds with 3 to 10 animals each in relatively small breeding area. That distribution could contribute to higher decreasing in size of Turopolje pig population due to outbreaks of infection diseases.

## CONCLUSIONS

Traditional Turopolje pig production system could be define as an extensive swine production system in which large areas of pasture and woodland are used (more than 3 ha/pig) with low investment in housing and feeding. As a main limiting factors in this system were obtained: outbreaks of infections diseases (brucellosis, swine fever, leptosirosis), low farowing index (1.0 to 1.33), small litter size (4.21 piglets born alive), high mortality of piglets in suckling period (up to 35%) and consequently low number of weaned piglets per sow per year (4.45). This could explain slow growth in size of Turopolje pig population since 1996 to 2009 (in total 42 vs. 156 breeding animals in year 1999 vs. 2009, respectively). In order to changes of that state urgent development of health management program and improving in production system (housing and feed supplementation in suckling period) as well as marketing of traditionally Turopolje pig meat products are needed.

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## Black Slavonian pig – a breed for extensive husbandry (A review)

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### **ABSTRACT**

*Black Slavonian (BS) pig is local Croatian breed originated in Slavonia region in the second half of the 19<sup>th</sup> century. Until the 1950s it was the most widespread breed in Slavonia, mainly used for the production of fat and meat products. Later, its population was drastically reduced and in 1990s the survival of the breed was endangered. In order to halt breed decline the State introduced protection measures which resulted in population increase; in 2008 there were 78 boars and 669 sows. The breed is well adapted for traditional outdoor keeping which includes the utilization of pasture and woodland with supplement of a small amount of grains. Litter size is low with ~ 1.5 farrowing per year per sow. The fattening abilities are also modest, with low daily gains and high share of fat in the carcass. However, the meat quality is good, with high content of intramuscular fat and high appreciation of its meat products. As a part of current trends of support of sustainable and traditional food production systems, it becomes important to preserve the production systems of BS pigs and their products. Development of marketing of niche products could be the best way for long-term preservation of breed.*

(Keywords: pigs, breeds, Black slavonian pig, production traits)

### **INTRODUCTION**

Black Slavonian (BS) pig is local Croatian breed which originated from Berkshire and Poland China breeds crossed with Black Mangalitsa. The breed was developed in Slavonia region in the eastern Croatia by Count Pfeiffer at the end of the 19<sup>th</sup> century. In the past this was the most numerous pig breed in Slavonia commonly used for production of fat and traditional meat products. However, over time it was largely replaced by more productive imported breeds and the population of BS pigs was drastically reduced. In 1996, the size of effective population was less than 20 and survival of the breed was endangered (Uremović *et al.*, 2000). The same year, Croatia signed the Biodiversity Treaty (CBD, 1992) and "A Survey of the State of Biological and Environmental Diversity of Croatia with Strategy and Protection Plan Action" was elaborated (Duzzp, 1999), as well as "A Program for Breeding up of the Black slavonian breed" (Uremović and Janeš, 2000). As a result of undertaken measures the breed decline was stopped. Moreover, its economical potential for the production of traditional products has been recently re-actualized (Uremović, 2004; Karolyi *et al.*, 2004; Karolyi *et al.*, 2007a). In recent years population of BS pigs rather increased; in 2008 it counted 78 boars, 50 young boars, 669 sows, 392 young sows and 1368 piglets (Croatian Agricultural Agency, 2009).

### Traditional production system

Traditional BS pig production is an outdoor, grazing system which includes utilization of natural resources of pasture and oak (*Quercus robur* L.) woodland with supplement of a small amount of corn seed or some other grains (~0.15 kg per head daily). Together with pasture, pigs consume foodstuff found on stubbles after the cereals (wheat, corn, and barley) have been harvested. The sows are conventionally kept in pens for farrowing littered with straw in eaves closed on three sides, one week before farrowing and after farrowing to weaning. There are on average ~1.5 farrowing per year per sow. During low temperatures piglets may be heated (i.e. with infra-red lamps). After the weaning, sows and piglets are kept in the open with the possibility of entering eaves. Usually, there are about twenty sows per ha. During rough winter months, the animals may also be kept inside piggery in the villages. The short period of pre-slaughter fattening with concentrated feed is common. In general, the breed is well adopted for outdoor keeping in conditions of continental climate due to its pronounced resistance, dark pigmentation and ability to consume large amounts of pasture (Uremović et al., 2003; Senčić et al., 2005).

### Production and carcass traits

As for the most of other local pig breeds, the production possibilities for BS pig are rather limited. Litter size is low with average number of live born and reared piglets per litter ranges between 6.3 and 7.4, and 5.7 and 6.6, respectively (Uremović et al., 2000; Senčić et al., 2001a; Uremović et al., 2003). Significant improvements in litter size traits were obtained by crossing BS sows with Duroc boar (Uremović et al., 2003). The fattening ability is also modest. Under the extensive production, daily gain in body mass from 27 kg to 106 kg was on average 478 g, with average share of meat in the carcass of 43% (Uremović et al., 2000). At fatteners of similar finally body weight but fed with the commercial feed mixture, Senčić et al. (2001b) determined average carcass meatiness of 38.5%. In some previous papers, even lower meatiness in the carcasses of similar weight (~80 kg) was reported: 28.6% (Petričević et al., 1988) and 28.5% (Kralik et al., 1988). In general, BS pigs are characterized by high accumulation of adipose tissue in the carcass similarly to other local breeds with low genetic potential for muscle development as Alentejano in Portugal (Freitas et al., 2007), Iberian in Spain (López-Bote, 1998), Cinta Sense in Tuscany (Pugliese et al., 2004) or Corsican breed (Coutron-Gambotti et al., 1998). For example, in heavy BS fatteners (at the age of about 18 months and average body weight of 160 kg) used for the processing of Slavonian kulen – a traditional dry sausage, the average depth of meat and fat above *m. gluteus* were nearly the same, 64 and 63 mm, respectively. In the carcasses of crossed white pigs (Large White×Swedish Landrace sired with Duroc) of similar age and weight the average depth of meat and fat were 73 and 30 mm, respectively. As a consequence, in comparison to modern white crosses, BS pigs have significantly lower utilization of main cuts (hams, back, shoulders and neck) (32.3 vs. 26.8%, P<0.05) and lower utilization of carcass (19.9 vs. 16.3%, P<0.05) for the production of traditional products like Slavonian kulen (Karolyi et al., 2004). These results however, were obtained after the prolonged period of pre-slaughter fattening in the piggery when pigs weight gain is mainly due to accumulation of fat. In the same experiment, the significant reduction of back fat was obtained in progeny of BS sows and Duroc boar. Results from traditional outdoor-low input system, reported by Senčić et al. (2005), showed the same shares of fat and muscle tissue (41%) in the carcasses of BS pigs reared until 12 months of age and average body weight of 130 kg. In the same experiment, significant improvements in the carcass

meatiness were obtained by crossing BS sows with Swedish Landrace boars (in F1 progeny the share of fat and muscle were 36.0 and 44.6%, respectively). Significant improvements of growth and carcass traits of F1 progeny in the outdoor production system reared up to the high body weights (~170 kg) were obtained also by crossing BS and Yorkshire breed (Uremović *et al.*, 2007), as well as in crossing with Duroc breed in fattening up to 120 kg of final weight (Luković *et al.*, 2007).

### **Welfare in the traditional production**

The knowledge about welfare issues in BS breed is limited. No research based on standard scientific measurements of animal welfare has been performed so far. However, it could be expected that in the traditional outdoor systems, both physical and mental well-being of the animals are good. The pigs are reared extensively, they spend most of their life-time outside on pastures and oak woods where they are able to exhibit foraging behavior and other natural instincts. It is well known, however, that outdoor farming is more often associated with parasite-related diseases (Hovi *et al.*, 2003). One of the recent no-drug recommendation methods of gastrointestinal nematode control in grazing animals is the consumption of plants rich in condensed tannin, as it has a direct toxic effect on parasites and/or on the parasite fecundity (Hoskin *et al.*, 2000). By grazing in oak woods, especially during season when alternative forage availability is scarce, BS pigs may consume huge amounts of tannin rich plant material. To research tannin protective ability, Salajpal *et al.* (2004) fed BS pigs three weeks before slaughter with acorn *ad libitum* (experimental group) or with concentrated feed (control). All examined pigs were reared (spring-autumn) on pasture utilizing natural resources and they naturally acquired parasite. It was found that oak acorn (*Quercus robur* L.) is a relatively tannin rich forage (65 g/kg of DM) and that its consumption can reduce total faecal egg count output (96.01%) in pigs infected with large roundworm (*Ascaris suum*) and other gastrointestinal parasites. It is concluded that the acorn grazing in the traditional BS pig production system may have a potential of aiding in the control of the gastrointestinal parasites and consequently may result in reduced need for anthelmintic treatment.

### **Stress resistance**

Physiological responses to stress in pigs can be assessed by measuring changes in plasma cortisol, glucose, lactate and/or other parameters like activity of the cellular enzymes creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) which are commonly used as indicators of stress affecting muscle damage (Fàbrega, 2002). There are some indications that the resistance to stress during pre-slaughter handling of BS pigs seems to be superior to modern pigs. In the comparison with modern white crosses, Black slavonian pigs had the lowest ( $P<0.05$ ) serum CPK and AST activity in the blood samples collected at exsanguinations, which may indicate their lower susceptibility to pre-slaughter stress (Karolyi *et al.*, 2004). Nevertheless, this single finding needs to be investigated in the future in more detail (e.g. cortisol level).

### **Meat quality**

Regarding the meat quality of BS pigs, in some previous papers no prominent defects were reported (Petričević *et al.*, 1988; Kralik *et al.*, 1988). The average values of initial pH within 1 h post mortem ( $pH_1$ ) and final pH after 24 h post mortem ( $pH_{24}$ ) in longissimus muscle (LM) reported more recently were inside normal pork quality scope and ranged between 6.1 and 6.8, and 5.7 and 5.9, respectively (Senčić *et al.*, 2001b;

Senčić et al., 2005; Karolyi et al., 2004; Uremović et al., 2007; Karolyi et al., 2007b, Butko et al., 2007). The meat of BS pigs is visually darker and redder than the meat of modern pigs. The lightness (Cie  $L^*$ ) and redness (Cie  $a^*$ ) values of LM colour measured 24 h post mortem were 49.9 and 20.0, respectively (Karolyi et al., 2004). The water fixation ability assessed by compression method was between 4.0 and 4.5 cm<sup>2</sup> (Senčić et al., 2001b, Senčić et al., 2005, Butko et al., 2007), while measured by drip loss method it was 1.6% (Uremović et al., 2007).

In LM of BS pigs, Salajpal et al. (2007) found a higher ( $P<0.01$ ) share (%) of red slow-twitch oxidative muscle fibres and fast-twitch oxidative-glycolytic muscle fibres in comparison to modern crossbred pigs of similar age, weight and keeping conditions. The authors also found lower ( $P<0.05$ ) diameter in red slow-twitch oxidative fibres and white fast-twitch glycolytic fibres in BS pigs. These results are in accordance with findings of Rahelić and Puać (1981) who suggested that the diameter of LM muscle fibres in pigs increases with selection along with decrease of share of red fibres and increase of share of white fibres. The indication of lower stress susceptibility of BS pigs mentioned previously could also be related to higher muscle oxidative capacity in comparison to modern pigs.

The most distinctive characteristic of meat of BS pig in comparison to today's commercial pork is particularly high content of intramuscular fat (IMF), in average 6 to 8% (Petričević et al., 1988; Senčić et al., 2001b; Senčić et al., 2005; Karolyi et al., 2007b). Many local pig breeds in extensive production systems show similar degree of intramuscular fat accumulation. This particular ability is obviously favoured by genotype x environment interaction pointed out by Lebret (2007); where the slower growth rate and higher final body weight and age together with specific diet based on pasture, acorn and other natural foodstuffs allow pigs the positive expression of genetic potential for deposition of fat into the muscle. Besides this, the compensatory growth usually present during energetic abundant diet in autumn after the period of scarce diet during summer, may favour fast increase in adipose tissue mass and deposition of large amount of lipids into the muscles in breeds with low genetic potential for muscle growth (Acciaioli et al., 2002; Coutron-Gambotti et al., 1998). Butko et al. (2007) reported approximately 1% higher ( $P<0.01$ ) share of IMF in LM of BS pigs from traditional outdoor system in comparison to animals kept inside and fed by commercial mixture. The higher share of IMF in pigs from outdoor production systems was also reported in other local breeds, e.g. Cinta Sense (Pugliese et al., 2004) and Corsican pig (Coutron-Gambotti et al., 1998). These findings could be related to differences in growth rate as animals raised under the extensive production usually reach the final body weight with considerably higher age and degree of development of adipose tissue (Pugliese et al., 2004).

### Traditional feedstuffs and fatty acids

Natural resources feeds like grass are a good source of alpha linolenic acid (ALA) (Muriel et al., 2002), an n-3 PUFA which is believed to confer benefits to human health. The influence of feedstuffs in the traditional production system of BS pigs, which include the pasture in spring and stubbles in late summer and autumn, on fatty acid (FA) profile of meat and back fat has been investigated by Uremović et al. (2007). The authors determined the average share of saturated FA (SFA), monounsaturated FA (MUFA) and PUFA in LM and back fat of 37.5, 56.9 and 5.5%, and 35.0, 47.6 and 16.7%, respectively. Investigating the effects of breed (BS vs. BS×Duroc) and rearing system (indoor vs. outdoor) on FA profile of *M. semimembranosus*, Luković et al. (2009) found lower SFA and MUFA and higher PUFA and ALA share in BS pigs. The share of

ALA in muscle did not differ between rearing systems probably due to fact that indoor kept pigs were also supplemented with mowed green mass. The indication of beneficial effects of traditional foodstuffs was found when BS pigs were fed with oak acorn (*Quercus robur* L.) instead of concentrate feed during finishing pre-slaughter fattening (Karolyi *et al.*, 2007b). Feeding acorn *ad libitum* three weeks before slaughter significantly increased the content of ALA in the LM in comparison to concentrate fed group (0.37 vs. 0.12, respectively; expressed as percent of total fatty acid methyl esters,  $P<0.01$ ). These results imply that traditional feeding of BS pigs with acorn may increase the content of n-3 PUFA in meat what could be beneficial from consumer's health point of view.

## CONCLUSIONS

It could be concluded that *in-situ* conservation of local BS pig so far has been successful. However, long term re-establishment of breed must be considered on economical basis by improving its traditional production system and by developing niche market products. The restoration of the traditional link between the breed and its typical products may represent a key tool to achieve these objectives. In this way, it is reasonable to start breeding the BS pigs again for the production of value added traditional products which could be, by itself, the best way for long-term preservation of breed. Additional research is needed on genetics, meat and products quality. The most interesting trait, the high IMF content, should be related, both to sensory and nutritional properties of meat and products. Influence of traditional feedstuffs on quality and FA profile of meat as well as potential benefits on consumer health should also be researched. If there are such benefits, they should be promoted in the public and in the market.

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## Improvement of animal welfare in lactating sows reared outdoor in Mediterranean area

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### **ABSTRACT**

*The project focused on studying the behaviour of free range sows, to evaluate the level of utilization of different areas inside the paddock (hut, shaded area and wallow) under different climatic conditions. In the study has been also assessed the sows preference towards a certain type of hut. The behaviour of 23 lactating sows, kept outdoors in individual farrowing paddock, were observed in 12 months starting from August in order to monitor it under different climatic conditions. Activities and areas in which these actions were performed were recorded from 8.30 a.m. to 4.30 p.m. for a total of 31 days. Considering the thermal requirements of pigs, the days of observation were divided into three categories: cold ( $\leq 15$  °C), optimal (from 15 to 25 °C) and hot (over 25 °C). The white hut seem to be preferred by the sow even if the inner temperature is not different from one of the gray. This preference could be due to the length of both sides which allow an easier transition from standing to lying down. Shaded area seems to be under-exploited, also under hot environment and this is likely due to the insufficient protection from sun radiation. The presence of the wallow seems to positively affect the welfare of sows during hot season anyway other systems of cooling such as sprinkler could be tested in order to reduce the heat stress in outdoor sows reared in the mediterranea area.*

(Keywords: outdoor, hut, wallow, environmental temperature, lactating sow)

### **INTRODUCTION**

Economic circumstances and concerns about animal welfare in intensive systems led to a rapid increase in outdoor production in North Europe (Edwards, 1994) and, specifically, interest in the production of free-range pigs (McGlone, 2000). Success of outdoor pig systems may depend on the details of the housing design, management, and location, including soil type and climatic conditions (Turner, 2002). Modern, intensive outdoor sows are farrowed in individual huts, and hut design is thought to be a crucial asset in piglets survival. Several farrowing hut designs are currently in commercial use with varying performance success. Research has focused on the shape of the hut (McGlone, 2000), the space requirement needs of the sow and the insulation status (Johnson, 2003). However, none of these studies was conducted in the Mediterranean area, where free-range farming is growing but the same area is characterized by high temperature during spring and summer. Temperatures that exceed 32 °C compromised the welfare status of lactating sows kept outdoors in summer unless they are assisted in thermoregulation (Johnson, 2008). The project focused on studying the behavior of free range sows, to assess the degree of utilization of different areas inside the paddock (hut, shaded area

and wallow) under different climatic conditions. Assessing whether the sows show a preference for different types of hut. The information gathered will be used as technical support for the development of new types of huts and eventually reorganization of spaces available in outdoor rearing system for sows.

## MATERIALS AND METHODS

The research has been conducted in a farm belonging to Veneto Agricoltura (extension service of Veneto Region) located in Ceregiano (RO) on a group of 23 lactating sows kept outdoor. Approximately 10 days before the estimated date of the farrowing, sows were moved from the gestation to an individual farrowing paddock fenced by a double electric wire and of the size of about 13×34 m. During the lactation period sows were fed twice a day with a feed formulated to meet the needs of this stage production. The piglets were free to move outside of the paddock, socializing with other litters and making the mixing of groups at the time of weaning less traumatic and stressful. Within each area there were two huts always accessible to the sow, we call them white and gray hut in relation to the color of the structure. The gray one, is widely used in north Europe, is made of wood and galvanized sheet and isn't insulated, while the white one is a prototype designed by engineers Veneto Agricoltura and is made in fiberglass and insulated with the intent to protecting sows from the heat. Both shelters had the same orientation so that the entrance were located downwind. Both were placed directly on the ground without any special floor while the straw was distributed on the floor to ensure more comfort to sow and the piglets. The gray hut was in English style with a form of semi-arch of maximum height of 1.10 m and the base size of 3 m in length and 1.5 m wide with a total area of 4.5 m<sup>2</sup>. Access to the shed was allowed by an opening of 80×90 cm located at the extremity of the wall to avoid the direct exposition of piglets to cold air currents. The opposite side has a small opening to ensure the air flow and to allow the operator to control the litter, especially near the farrowing time and without disturbing the sow (*Figure 1*).

**Figure 1**

**Gray hut, front view on the left and rear view on the right**



The white hut was in the shape of a real house and was an insulation structure of fiberglass with a layer of polyurethane foam inside. The basis of this structure measures 2×2 m<sup>2</sup> with an area available for the sow of 4 m<sup>2</sup> and this was slightly lower than the

reported for the other kind of hut. Even in this case the passage was not centrally located in the wall and its measure was 70×90 cm. On the back there was a window of 15×25 cm placed at 80 cm from the ground that can be closed by a sliding cover. The nest measure 145×30 cm and 25 cm high, with covered opening on the roof that allows easy inspection of the litter by the operator. A certain level of isolation of the nest can improve the microclimate for the piglets and is also an area protected from the crushing due to the lying down of the sow to breastfeeding (Figure 2).

**Figure 2**

**White fiberglass hut view on the left and side view with nest on the right**



In each paddock both types of huts had been presented. The sows had also a rest area equipped with shade net and a wallow to make a mud bath possible. In each paddock there was a nipple waterer for drinking water distribution.

The experimental measures had been mainly related to behavioural observations, carried out from 8.30 am to 4.30 pm for a total of 31 days chosen on the basis of the environmental temperature in different seasons, in order to obtain at least 10 days of observation in cold, optimal and hot environment. Each hour every sow had been observed for 15 minutes, using the "Scan Sampling" technique, with 1 min of scan interval (Martin and Bateson, 1993). For each sow in each sampling interval, the states of the animal (nursing, exploring, rooting, eating, drinking and resting) and area in which these activities were performed (white or gray hut, paddock, wallow, shaded area) had been recorded. Data collected had been processed in order to calculate the percentages of time engaged in this specific activities by each sow. In each day of observation, temperature and humidity inside and outside the huts at 8:30 a.m., at 1:30 p.m. and 5:30 p.m. had also been recorded.

Considering thermal requirements of pigs (Federation of Animal Science Society, 1999), the days of observation were divided into three categories: cold (average temperature less than 15 °C), optimal (temperature between 15 and 25 °C) and hot (temperature over 25 °C). The behavioral data collected were submitted with the statistical analysis system SAS (2001) to analysis of variance using and adopting a linear model which considered the categories of temperature, parity and their interaction.

## **RESULTS AND DISCUSSION**

The statistical analysis, did not show significant interactions between the main factors therefore this allows a separate discussion on the effect of parity and environmental temperature. In outdoor rearing systems, the presence of a hut dedicated to the protection of the litter is certainly an important element. The presence of the sow inside the hut is however conditioned by the distance from the farrowing (*Figure 3*). The lower use of the hut 1–2 days before farrowing can be related to the considerable locomotion showed by the sow due to the research of material such as straw, grass, twigs needed for the nest building. Unlike the sows increased their presence in the hut in the first week after farrowing, when the piglets require more intensive protection and need to be fed frequently. Considering the type of hut it is possible to observe that the white one is always preferred to the gray one. The use of the gray hut is unaffected by weather conditions while for the white hut it is possible to observe a significant decrease in its use in hot environment (*Figure 4*). Considering into account that temperature and humidity inside the two structures did not show statistically significant differences, the preference towards the white hut leads to the hypothesis that other factors are behind this decision, in particular the shape of the hut and the location of the entrance.

It is likely that the white structure, although the total area available is 50 cm<sup>2</sup> less than the gray one, allows the sow to lie down on both sides of the hut so she is able to rest and relax more comfortably during breastfeed. At the same time the sow can always keep the head turned outwards. The possibility to see and control what is happening outside the hut is essential for the sow in order to develop all possible defense strategies of the offspring. The size of the white hut is important in determining the preference of sows (*Figure 5*). Multiparous sows usually are heavier than primiparous (250 vs 150 kg) and therefore they prefer white structure because they have more space available, which allows an easy transition from station to recumbent and vice versa. In the gray hut, which has a rectangular plan, the size of the short side seems to be the main constraint to the correct execution of these movements. Climatic conditions have a strong effect on the use of different part of the paddock (*Figure 6*), especially since the pig has some difficulties to implementing the processes of thermoregulation. In particular if we consider the effect of temperature on the activities undertaken by the sow, it can highlight how the wallow has been primarily used in high temperature conditions as a strategy to overcome the heat stress, in agreement with results obtained by *Bull et al.* (1997). Lying down on cooler surfaces can dissipate the heat more efficiently by conduction rather than by convection (ventilation) or by evaporative cooling ( During hot days the visiting frequency of the wallow is doubled than those with optimal temperature and 5 times higher than in days with temperatures is defined 'cold'. It should be emphasized that the use of wallow is mainly concentrated in the central phase of the day between 11:30 am and 3:30 pm, time interval in which there were obviously higher temperatures. The exploitation of the shaded area in warm weather has been more limited than the use of the wallow, probably because it is less effective in the control of heat stress.

Regardless of age of the sow, rooting and grazing were mainly performed in the afternoon and they were enhanced by low environmental temperatures (*Figure 7A*). In the same conditions a consequent reduction of the resting time had also been observed (*Figure 7B*).

Figure 3

Frequency of use of the hut in relation to the distance from farrowing

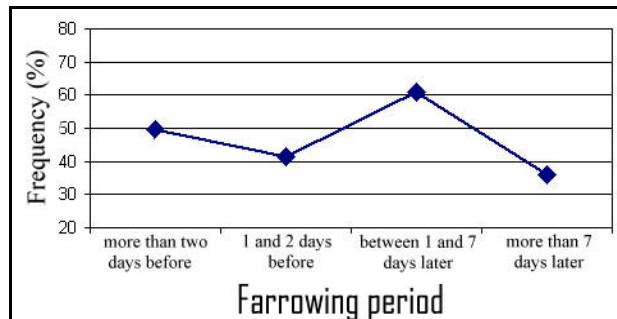


Figure 4

Frequency of use of two types of hut in relation to environmental temperature

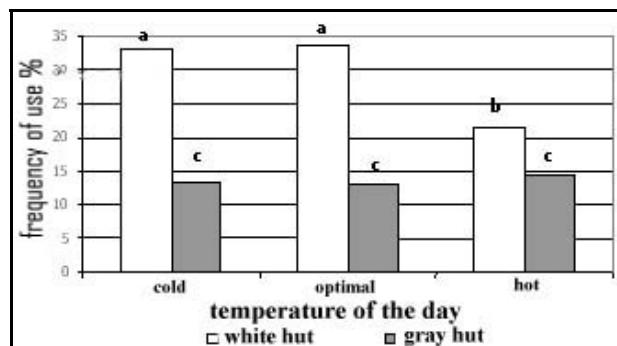
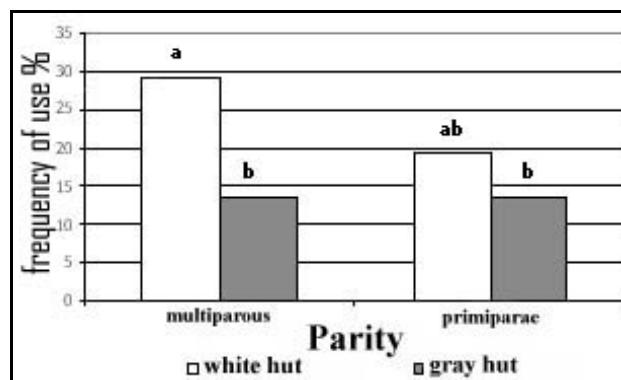


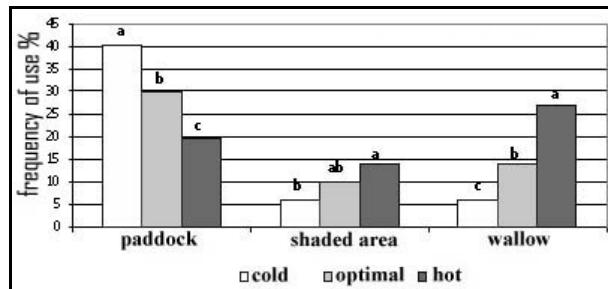
Figure 5

Effect of the sows parity on the use of white or gray hut



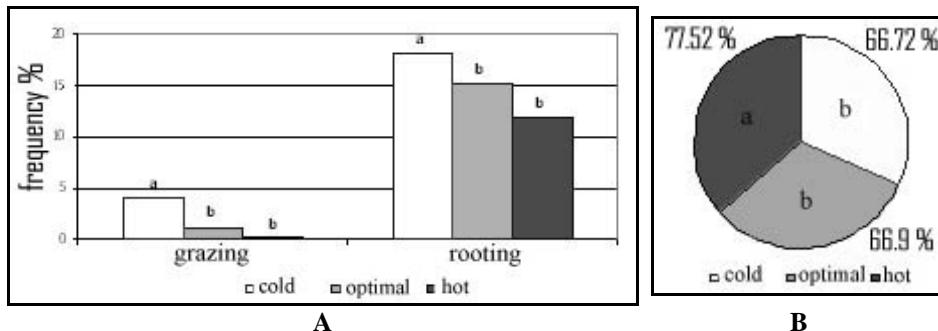
**Figure 6**

**Effect of environmental temperature on the utilization of different part of the paddock**



**Figure 7**

**Effect of environmental temperature on activities (A) and rest (B) performed by the sows**



## CONCLUSIONS

The length of both sides of the white hut should allow an easier transition from standing to lying down and vice versa. In addition, in order to decreasing the stress level of the sow, she should always be able to see what is happening outside. Shaded area seems to be under-exploited, under hot environmental conditions as well and this likely due to the insufficient protection from sun radiations and this required more specific studies aimed to identify the best shading material. The presence of the wallow seems to positively affect the welfare of sows during the hot season, anyway other systems of cooling such as sprinkler could be tested in order to reduce the heating stress in sows reared outdoor in the mediterranea area.

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## Variability in carcass composition of pigs during growth

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### **ABSTRACT**

*The purpose of this study was to investigate variability in carcass composition of fattening pigs during growth. The research was conducted on 60 three way hybrid pigs between 49 and 215 days of age. For the purpose of growth measurement pigs were weighed at 7 days interval. After each 21 days, four pigs (2 barrows and 2 gilts) were selected for slaughter according to average live weight. Subsequent to slaughter, right side of pig carcasses was dissected according to Weniger (1963). Statistical analysis showed that average live weight increases with age in the sigmoid manner. The average daily gain during the trial was 550 g (562 g for barrows, and 537 g for gilts), while feed conversion was 3.5 kg. The highest share of muscle was achieved at 125 days of age for barrows and 146 days for gilts. The intramuscular fat content was higher in barrows than in gilts throughout the whole experiment time. Share of bone tissue in investigated pigs constantly decreases, but after 125 days of age it remains at the same level.*

(Keywords: pigs, carcass composition, growth characteristics)

### **INTRODUCTION**

Growth of certain body parts or tissues such as muscle, fat, skin or other has been of interest for animal scientists a long time. Although the fat tissue accumulates during the whole lifetime, intensive period begins in the second part of fattening. At this time accumulation of muscle tissue decreases. The moment of this event depends on genetic structure of the animal. The investigation of such matters resulted in useful knowledge which led to more effective selection on daily gain, increased muscle content and decreased fat content in pig carcasses (Wiseman *et al.*, 2007). Reeds *et al.* (1993) showed that during the last 50 years live weight of the pig at the same age increased for 20%, muscle content increased for 86%, while bone and fat content decreased for 11% and 29%, respectively. Having in mind that better efficiency in utilization of growth characteristics in pigs can be of benefit for the pig producers, the aim of this research was to investigate variability in carcass composition of pigs originating from Bataille breeding program during growth.

### **MATERIALS AND METHODS**

The research was conducted on 60 (30 barrows and 30 gilts) three way hybrid pigs (Bataille breeding program) aged between 49 and 215 days. Four piglets (2 male and 2 female) per sow were included in the investigation. Pigs were housed in the same conditions and fed the same diet. The animals were fed *ad libitum* during the fattening period. Feeding scheme is shown in *Table 1*.

**Table 1****Feeding scheme**

<b>Grower (&lt;25 kg)</b>	<b>Finisher (&gt;25 kg)</b>
<i>ad libitum</i>	<i>ad libitum</i>
13.3 MJ ME	13.6 MJ ME
19.6% crude protein	17.4% crude protein

For the purpose of growth measurements pigs were weighted every 7<sup>th</sup> day during the experiment. Every 21<sup>th</sup> day four pigs were selected for the slaughter according to average live weight (LW). These animals were slaughtered at Vajda d.d. slaughterhouse and total dissection according to *Weniger* (1963) on the right side of the carcass was performed. For this part of experiment 15 barrows and 15 gilts were used. Statistical analysis was performed using SAS/STAT for Windows platform (SAS Inst. Inc., 2003, Version 9.1).

## RESULTS AND DISCUSSION

### Live weight

During the experiment, at the age of 82, 110 and 131 days, 3 gilts were excluded because of death or health issues. The average LW of animals at the beginning of study was approximately 22 kg, and 137 kg at the end. *Figure 1* and *Figure 2* show the increase of LW over time. It can be noticed that average LW of pigs during the growth resembles a sigmoid shape: low growth rate at the beginning of fattening is followed by an increase at the certain age (inflection point) and, at the end it decreases. During the research a homogeneous data structure at the beginning of experiment can be noticed, but later, with the increase in age and the decrease in number of investigated animals, variability markedly increased. *Solanes et al.* (2005) reported similar pattern in Batalle hybrids, although lower LW was achieved (average LW of 102.4 kg in 179.6 days). From *Figure 2* it can be seen that barrows obtained higher LW than barrows for the same period of time. *Reixach et al.* (2008) reported similar results regarding the change of LW in barrows. At the age of 200 days Landrace×Yorkshire hybrids had an average LW of 136.04 kg, Duroc pigs had 124.42 kg, Iberian×Duroc hybrids had 122.22 kg and double line Duroc pigs had 121.49 kg. Similarly, *Ball* (2000) reported that average age of 157.9 days is required to achieve 100 kg of LW in Duroc breed. *Šalehar et al.* (1995) investigated the differences in LW among several breeds. The authors reported that at the age of 90 days average LW of Duroc boars was 31 kg, which is considerably less than the results obtained in this study. However, in later stage (about 210 days) of their research Duroc boars obtained higher LW than the animals included in this research.

### Daily gain

The average daily gain during the trial was 550.00 g (562.00 g for barrows, and 537.00 g for gilts), which was less than those reported by *Šalehar et al.* (1995); *Ellis et al.* (1996) and *Ball* (2000). *Figure 3* shows variability in daily gain of gilts and barrows. Average daily gain between weighing was 0.66 kg, with a minimum of -0.94 kg and maximum of 1.71 kg.

Figure 1

**Distribution of pig live weight at different age**

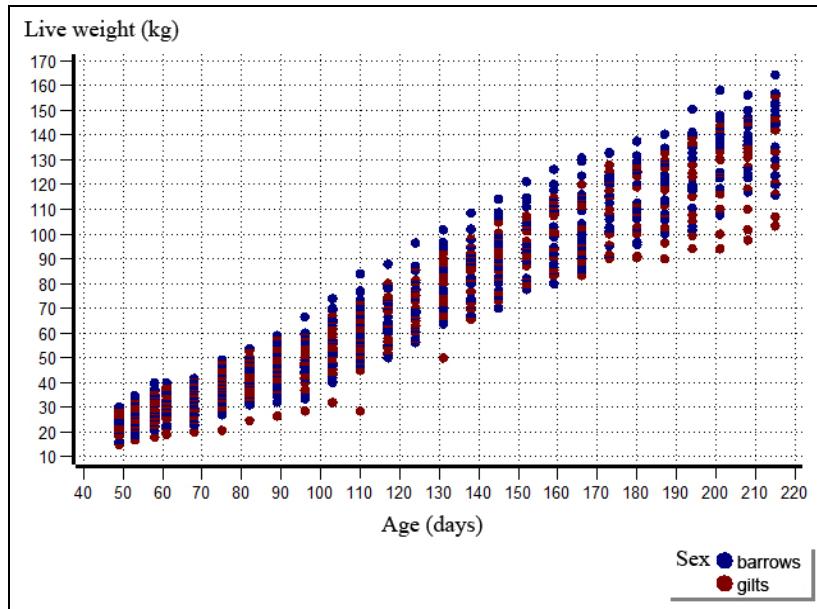
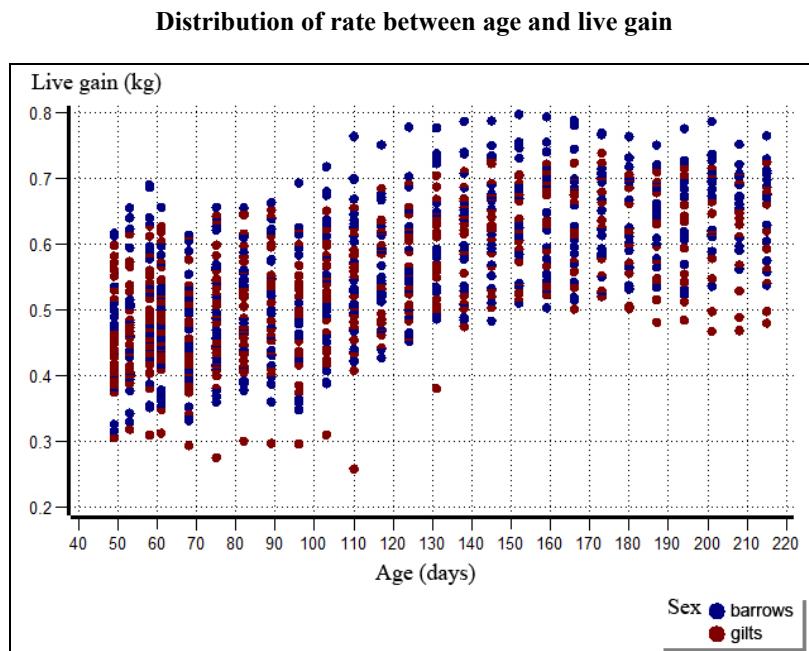


Figure 2

**Average live weight of investigated pigs at different age**



**Figure 3**

During the experiment a total of 16,635.00 kg of feed has been used for "ad libitum" feeding regime. Summing up the total final LW and putting it in relation with consumed feed, 3.5 kg of feed per kilogram of gain was estimated. *Kanis et al.* (1990) reported similar results for the feed conversion. The authors reported that Duroc pigs consumed 3.37 kg of feed per gained kilogram during the test between 60 and 100 kg of LW, in contrast to Pietrain pigs which consumed 3.18 kg. Between 100 and 140 kg this consumption was significantly higher (4.45 kg, 4.71 kg), but throughout the whole experiment (60–140 kg) 3.89 kg of feed was consumed, i.e. 4.00 kg in average. *Affentranger et al.* (1996) reported that feed conversion for Duroc pigs weighing from 25 to 103 kg was 2.75 and 2.59 kg for Pietrain, while *Šalehar et al.* (1995) reported conversion of 2.82 kg in Duroc boars.

### Tissue growth

The results of dissection of pig carcasses at different age are shown in *Figure 4* and *Figure 5*. It can be noticed that gilts obtained higher percentage of meat, fat, IMF and bones in the carcass during the growth. This trend was retained until the age of 188 days, after which a decrease of all tissues was recorded. Unlike gilts, barrows continued linear growth of all tissues. The muscle tissue increased proportionally with increase of pigs LW. Bone growth patterns are similar to the results reported by *Wagner et al.* (1999). Bones grew significantly slower compared to the muscles.

Share of tissues alters with the age and the increase of pig carcass weight. In this study, the highest percentage of muscle tissue in the carcass of gilts was achieved at the age of 146 days (67.18%), while in the barrows; the highest percentage of muscle (60.22%) was recorded at 125 days of age. Also, from that moment the significant fat accumulation in the barrows bodies begun.

Figure 4

**Share of meat, fat, intermuscular fat (IMF) and bones in the carcasses of barrows**

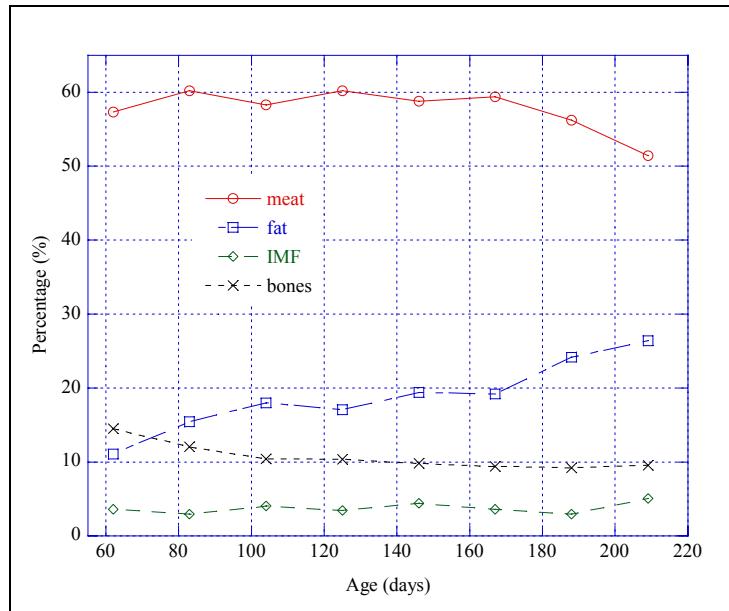
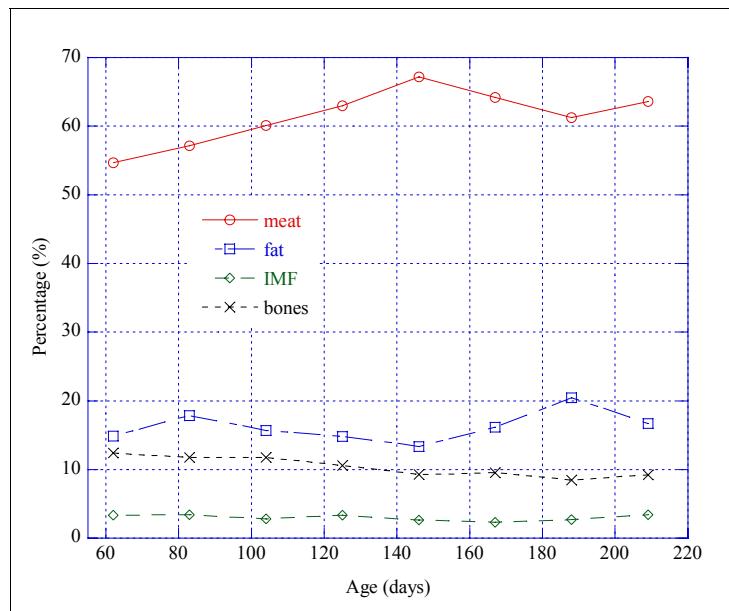


Figure 5

**Share of meat, fat, intermuscular fat (IMF) and bones in the carcasses of gilts**



On the basis of research on the similar samples of pigs at 100 kg of LW, *Cilla et al.* (2006) found the lean share from 53.1% to 57.6%. This is considerably lower than lean share established in this study. It was stated in many studies that Duroc pigs have a very favorable growth characteristics. Nevertheless, research of *Edwards et al.* (1992) and *Affentranger et al.* (1996) showed that Duroc pigs achieved lower growth performance than in the present study. Possible explanation for these results could be existence of several different Duroc "types". Experts in *Seleccion Batalla* (2006) breeding company reported the content of 1.83% of intramuscular fat in their studies on gilts with 100 kg of LW, which is much higher than IMF content in gilts presented in this study (1.15%). Results of *Reixacha et al.* (2008) indicate the existence of genetic variation in fat and intramuscular fat content, which can depend on the age at which the sample was taken. Therefore, the authors propose to develop selection criteria based on the final live weight. According to *Ball* (2000), differences within and among the pig breeds enable the manufacturers a suitable response to market demands by specific production. For example, the author in his study confirmed higher marbling score in pig carcasses of Duroc breed compared to other breeds.

## CONCLUSIONS

Average live weight of animals increases with age following the sigmoid pattern. The average daily gain during the research period in barrows was higher than in gilts. Feed conversion in the observed period was 3.5 kg. By applying the method of total dissection at regular intervals during the research it was found that the highest percentage of muscle tissue in the body of gilts was achieved at 146 days of age, in contrast to the barrows, where the highest incidence of muscle was determined at the age of 125 days. Thereafter, a significant accumulation of fat tissue in barrows was noticed during the dissection of selected carcasses. Throughout the entire period of study the percentage of intramuscular fat in the barrows was higher than in gilts. Share of bone tissue in barrows and gilts at 125 days of age decreases and then remains at the same level until the end of the study. As a practical consequence of this study it can be recommended to take into consideration the differences between genders in investigated growth characteristics when decisions on fattening period and slaughter weight/age are made.

## ACKNOWLEDGEMENTS

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## Influence of pig carcass weight on distribution of tissues in main parts

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### ABSTRACT

*The present study was performed on 241 swine carcasses were divided into 3 weight groups according to the warm carcass weight (light group: 60–80 kg; medium group 80–100 kg and heavy group 100–120 kg). Using the measures of backfat and muscle thickness lean meat percentage of pig carcasses was predicted by the equation for TP method prescribed in Croatia. After 24 hours of cooling, right sides of the carcasses were dissected according to EU referent method into four main parts (ham, shoulder, loin and ribs) and further into major tissues (muscle, subcutaneous fat with skin, intermuscular fat and bones). Obtained data was statistically analysed and differences between the weight groups were established for the carcass traits and tissue composition of the main parts. It was concluded that although significant differences existed in backfat and muscle thickness between the pig carcass weight groups, lean percentage predicted or established by dissection these carcasses was not significantly altered. Moreover, the percentages of tissues dissected from the main parts rarely differed significantly between the weight groups of carcasses. This is especially important for the percentage of muscle tissue in the main parts which increased only in ham. As a general conclusion it can be stated that increasing pig carcass weight does not necessarily have to impair other carcass traits or tissue distribution in the main parts.*

(Keywords: pigs, carcass weight, dissection, carcass composition, main parts)

### INTRODUCTION

Beside the age, genotype, sex, feeding regime and other sources of variance, it is well known that carcass traits can differ between the pigs regarding the slaughter weight (Cisneros *et al.*, 1996; Candek-Potokar *et al.*, 1997; Correa *et al.*, 2006). Data collected from Croatian slaughterhouses in past ten years show trend of increasing the slaughter weight, from 99.14 kg in 1999 up to 104,59 kg in 2008 (*Annual report of Croatian Agricultural Agency*, 2008). During that time, similarly to some other countries, the lean percentages in the carcasses of slaughtered pigs also increased resulting in heavier carcasses (Bachelka *et al.*, 2005; Kušec *et al.*, 2009). In fact, Correa *et al.* (2006) suggested that pigs can be slaughtered at heavier weights without compromising carcass quality. However, some pig producers tend to slaughter their pigs at lower live weights in the belief that they will achieve higher scores of carcass grading. In this light, the objective of present paper is to investigate the effect of hot carcass weight on the distribution of main tissues in the joints after thorough dissection according to EU reference method (Walstra and Merkus, 1996).

## MATERIALS AND METHODS

This study was performed on 241 swine carcasses selected on the basis of backfat measures obtained by "TP" method, approved in Croatia and slaughtered in slaughterhouses across Croatia during several dissection trials in past 4 years. On the basis of carcass weight the carcasses were divided into 3 weight groups as follows; light group weighted between 60 to 80 kg, medium group 80 to 100 kg and heavy group was between 100 and 120 kg. Before dissection, necessary measures for lean percentage prediction by "two points" (TP) method were obtained as follows: lumbar muscle thickness – Mdt (mm); measured as the shortest connection between the cranial end of the lumbar muscle and dorsal edge of the vertebral canal, and fat thickness – Sdt (mm), measured as the minimum thickness of subcutaneous fat (with skin) at the split of the carcass, above *m. gluteus medius*. Using these measures lean percentage of pig carcasses was predicted by the equation prescribed for TP method in Croatian legislation (NN 40/2007). One day after the slaughter, right sides of the carcasses were dissected according to EU referent method (Walstra and Merkus, 1995). Four main parts (ham, shoulder, loin and ribs) were dissected into muscles, bones, intramuscular and subcutaneous fat with skin and weighted. The reference lean percentage was expressed as prescribed by valid EU regulation (EC No. 1197/2006). The data were analysed using STATISTICA (data analysis software system), version 8.0 (StatSoft, Inc., 2007).

## RESULTS AND DISCUSSION

The measures relevant for methods of estimation of the percentage of muscle tissue in pig carcasses acquired on a slaughter line are shown in *Table 1*. The most of the randomly chosen pig carcasses belonged to the group with medium carcass weight (80–100 kg). Statistically significant differences were found between the groups in measures of backfat and muscle thickness, but not in estimated or dissected lean percentage. The lightest group had significantly lowest backfat thickness; while there were no significant differences between the other two groups of carcasses in that respect. It is obvious that lean percentage was quite similar in all investigated carcasses, irrespective of carcass weight. There is common belief between the pig producers that lowering the carcass weight results in the increase of lean percentage due to the lowered backfat thickness. Results presented in this study could not support that idea. The reason for this can be in the fact that significant increase in backfat thickness was followed by proportional increase in the thickness of muscle measurements in the heavier groups of carcasses which differed among all investigated groups.

*Table 2* presents the composition of the hams in absolute and relative terms of dissected tissues components. Naturally, heaviest carcasses had the heaviest hams; although in relative terms the difference between the groups was not statistically significant. Regarding the muscle tissue, significant difference was found between all investigated groups; the heaviest group produced the highest amount of muscle tissue, followed by the medium and light group. However, in relative terms, the lightest group had higher proportion of lean than the hams in other two groups. It can be observed that in the lightest group of carcasses, subcutaneous fat and IMF values were significantly lower than in other two groups in absolute and relative terms. The hams from the heavy group of pig carcasses had significantly more bones in absolute terms, comparing to other two groups, but bone percentage between the groups did not differ.

In *Table 3*, the differences in loin composition between the weight groups of carcasses is presented. Significant differences were found between all investigated groups in loin weight, the weight of muscle tissue and fat.

**Table 1**

**Least square means and standard errors calculated for the on-line measures, predicted and dissected lean percentage in investigated groups of pig carcasses**

Trait	Light (n=76)		Medium (n=128)		Heavy (n=37)	
	LS mean	S.E.	LS mean	S.E.	LS mean	S.E.
Carcass weight, kg	73.08 <sup>a</sup>	0.62	88.23 <sup>b</sup>	0.48	107.76 <sup>c</sup>	0.89
Sdt (mm)	13.04 <sup>a</sup>	0.64	15.92 <sup>b</sup>	0.49	18.76 <sup>b</sup>	0.92
Mdt (mm)	67.11 <sup>a</sup>	0.73	71.49 <sup>b</sup>	0.56	77.11 <sup>c</sup>	1.05
Estimated leanness (%)	57.74	0.54	56.66	0.42	55.67	0.78
Dissected lean (%)	55.06	0.68	54.43	0.52	54.60	0.97

Different superscripts within the row mean significant difference (P<0.05)

**Table 2**

**LS means and standard errors calculated for the dissected components of ham in investigated groups of carcasses**

Trait	Light (n=76)		Medium (n=128)		Heavy (n=37)	
	LS mean	S.E.	LS mean	S.E.	LS mean	S.E.
Ham (kg)	9.08 <sup>a</sup>	0.14	10.70 <sup>b</sup>	0.11	13.03 <sup>c</sup>	0.21
Ham (%)	30.64	2.66	26.65	2.05	24.95	3.82
Muscle tissue (kg)	6.60 <sup>a</sup>	0.13	7.47 <sup>b</sup>	0.10	9.00 <sup>c</sup>	0.18
Muscle tissue (%)	73.03 <sup>a</sup>	0.67	70.07 <sup>b</sup>	0.52	68.94 <sup>b</sup>	0.96
Subcutaneous fat (kg)	1.54 <sup>a</sup>	0.05	2.04 <sup>b</sup>	0.04	2.57 <sup>c</sup>	0.08
Subcutaneous fat (%)	17.08 <sup>a</sup>	0.53	19.22 <sup>b</sup>	0.41	20.07 <sup>b</sup>	0.75
IMF (kg)	0.29 <sup>a</sup>	0.02	0.39 <sup>b</sup>	0.01	0.54 <sup>c</sup>	0.02
IMF (%)	3.23 <sup>a</sup>	0.13	3.67 <sup>b</sup>	0.10	4.18 <sup>b</sup>	0.18
Bones (kg)	0.71 <sup>a</sup>	0.06	0.81 <sup>a</sup>	0.05	1.10 <sup>b</sup>	0.09
Bones (%)	7.92	0.46	7.57	0.36	8.48	0.66

Different superscripts within the row mean significant difference (P<0.05)

Heavy group of carcasses had significantly more kilograms of bones in loins than both other groups, but in relative terms the situation is reversed. In relative terms there were no differences between the groups in the shares of loin in the carcass, intermuscular fat and muscle tissue in the loins. The percentage of IMF was the lowest in the group of light carcasses. Dissection of the loins of the heavy group of pig carcasses resulted in significantly higher amount of the bones in absolute terms than in other two groups; while the percentage of bones in the loin differed significantly between all three groups, being the highest in the light group of pig carcasses, followed by medium and heavy group.

The differences in composition of shoulder between the investigated groups of pig carcasses are shown in *Table 4*. The weight of shoulder significantly differs among the groups. Increase of warm carcass weight of pigs resulted in significant increase in the weight of dissected muscle tissue from the shoulder. However, percentage of muscle in this part was unaffected by the carcass weight of slaughtered pigs. Significantly lower amount of subcutaneous fat was dissected from the shoulders of the light group of pig carcasses; in the relative terms the difference between the groups was not significant.

Regarding the intermuscular fat, higher amounts were found in shoulders of the heavy group of pig carcasses when compared with the other two. When relative proportions were analysed it was found that lowest percentage of IMF in the shoulder had the medium group of carcasses, which differed significantly only from the heavy group. The amount of bones differed significantly between the groups, but the lowest percentage of bones was found in the shoulders from heavy group of pig carcasses, while other two groups did not differ in that respect.

**Table 3**

**LS means and standard errors calculated for the dissected components of loin in investigated groups of carcasses**

Trait	Light (n=76)		Medium (n=128)		Heavy (n=37)	
	LS mean	S.E.	LS mean	S.E.	LS mean	S.E.
Loin (kg)	5.36 <sup>a</sup>	0.09	6.59 <sup>b</sup>	0.07	7.90 <sup>c</sup>	0.13
Loin (%)	18.66	1.88	16.57	1.45	15.15	2.69
Muscle tissue (kg)	3.28 <sup>a</sup>	0.07	3.88 <sup>b</sup>	0.05	4.64 <sup>c</sup>	0.10
Muscle tissue (%)	61.32	0.78	59.00	0.60	58.48	1.12
Subcutaneous fat (kg)	1.17 <sup>a</sup>	0.06	1.62 <sup>b</sup>	0.04	2.11 <sup>c</sup>	0.08
Subcutaneous fat (%)	21.65 <sup>a</sup>	0.77	24.52 <sup>b</sup>	0.60	26.91 <sup>b</sup>	1.11
IMF (kg)	0.22 <sup>a</sup>	0.02	0.31b <sup>b</sup>	0.01	0.34 <sup>b</sup>	0.03
IMF (%)	4.16	0.25	4.68	0.19	4.34	0.36
Bones (kg)	0.69 <sup>a</sup>	0.02	0.77b <sup>a</sup>	0.01	0.81 <sup>b</sup>	0.02
Bones (%)	12.87 <sup>a</sup>	0.21	11.80 <sup>b</sup>	0.16	10.26 <sup>c</sup>	0.30

Different superscripts within the row mean significant difference (P<0.05)

**Table 4**

**LS means and standard errors calculated for the dissected components of shoulder composition in investigated groups of carcasses**

Trait	Light (n=76)		Medium (n=128)		Heavy (n=37)	
	LS mean	S.E.	LS mean	S.E.	LS mean	S.E.
Shoulder (kg)	4.45 <sup>a</sup>	0.07	5.12 <sup>b</sup>	0.05	5.90 <sup>c</sup>	0.10
Shoulder (%)	14.93	1.25	12.78	0.96	11.35	1.79
Muscle tissue (kg)	2.89 <sup>a</sup>	0.05	3.25 <sup>b</sup>	0.04	3.75 <sup>c</sup>	0.08
Muscle tissue (%)	65.25	0.73	63.42	0.56	63.38	1.04
Subcutaneous fat (kg)	0.88 <sup>a</sup>	0.04	1.10 <sup>b</sup>	0.03	1.24 <sup>b</sup>	0.05
Subcutaneous fat (%)	19.82	0.64	21.52	0.49	20.97	0.92
IMF (kg)	0.23 <sup>a</sup>	0.01	0.25 <sup>a</sup>	0.01	0.34 <sup>b</sup>	0.02
IMF (%)	5.25 <sup>ac</sup>	0.19	4.83 <sup>ab</sup>	0.14	5.71 <sup>ac</sup>	0.27
Bones (kg)	0.48 <sup>a</sup>	0.01	0.52 <sup>b</sup>	0.01	0.58 <sup>c</sup>	0.01
Bones (%)	10.90 <sup>a</sup>	0.17	10.23 <sup>a</sup>	0.13	9.94 <sup>b</sup>	0.24

Different superscripts within the row mean significant difference (P<0.05)

The composition of the ribs of investigated groups of pig carcasses is shown in *Table 5*. The weight of this part is significantly increasing with the increase of whole carcass weight, as well as the amount of subcutaneous fat, IMF and bones in absolute terms.

Significantly lower amount of muscle tissue was found in the ribs of the light group than in other two groups which did not differ in that respect. In relative terms, only the percentage of bones dissected from the ribs differed significantly between all weight groups of pig carcasses; other components of the ribs from pig carcass groups did not differ in relative terms.

**Table 5**

**LS means and standard errors calculated for the dissected components of the ribs in investigated groups of carcasses**

Trait	Light (n=76)		Medium (n=128)		Heavy (n=37)	
	LS mean	S.E.	LS mean	S.E.	LS mean	S.E.
Ribs (kg)	3.13 <sup>a</sup>	0.07	4.06 <sup>b</sup>	0.05	5.20 <sup>c</sup>	0.10
Ribs (%)	10.70	1.04	10.17	0.80	9.98	1.48
Muscle tissue (kg)	1.78 <sup>a</sup>	0.11	2.31 <sup>b</sup>	0.09	2.73 <sup>b</sup>	0.16
Muscle tissue (%)	57.41	3.39	58.13	2.61	52.53	4.86
Subcutaneous fat (kg)	0.85 <sup>a</sup>	0.05	1.23 <sup>b</sup>	0.04	1.61 <sup>c</sup>	0.07
Subcutaneous fat (%)	26.72	0.98	29.86	0.75	30.87	1.40
IMF (kg)	0.28 <sup>a</sup>	0.02	0.38 <sup>b</sup>	0.02	0.53 <sup>c</sup>	0.03
IMF (%)	8.81	0.46	9.25	0.36	10.14	0.66
Bones (kg)	0.22 <sup>a</sup>	0.00	0.25 <sup>b</sup>	0.00	0.28 <sup>c</sup>	0.01
Bones (%)	7.06 <sup>a</sup>	0.12	6.25 <sup>b</sup>	0.09	5.37 <sup>c</sup>	0.18

Different superscripts within the row mean significant difference (P<0.05)

The results from the present study generally confirm the pattern of increasing mass of different carcass joints and the belonging tissues together with weight of an animal as shown by growth many studies (Davies and Kallweit, 1979; Gu *et al.*, 1992; Kouba *et al.* 1999). Similar investigation to the one in the present study was performed by Vališ *et al.* (2008). They analysed lean meat content within the four carcass weight intervals. Generally, it was confirmed that the lean meat content was reduced with increasing carcass weight. Significant differences were found between the intervals up to 94.9 kg and above 95 kg which were in agreement with the studies of Matoušek *et al.*, (2001) and Pulkrabek, (2003). In addition, they found that the contribution of main cuts also significantly decreased in heavier carcasses. Authors suggested that with increasing carcass weight, the contribution of different cuts to the total lean meat content is reduced at the same rate. The results of these authors cannot be supported by the present study since the reduction in lean percentage with increasing carcass weight was observed only in ham.

## CONCLUSIONS

On the basis of the results presented in this study it can be concluded that although significant differences existed in backfat and muscle thickness between the pig carcass weight groups, lean percentage predicted or established by dissection these carcasses was not significantly altered.

Although the main parts and tissues increased their weight in respect to the pig carcass weight group, the percentages of those rarely significantly differed between the weight groups of carcasses. This is especially important for the percentage of muscle tissue in the main parts which increased only in ham. As a general conclusion it can be

stated that increasing pig carcass weight does not necessarily have to impair other carcass traits or tissue distribution in the main parts.

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## Body weight of hinds as a stability factor in red deer (*Cervus elaphus*, L.) population

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### ABSTRACT

During the hunting season 2007/2008 on the government hunting-ground XIV/9 Podunavlje-Podravje (Danube area, Croatia), 67 hinds were examined. We have identified net weight – weight of the body in cold-storage, gross weight – calculating method, pregnancy, number and sex of the fetuses, as well as body weight and length of the fetus. The overall pregnancy rate was 75% (n=67). Among them 48% of the yearlings (n=19) were pregnant with 65,88 kg average net weight, however those of non pregnant weighted 51,60 kg respectively. 85% of the three-year old or older hinds was fecundated, and their average net body weight was 71,07 kg, whereas for the unfecundated hinds the body weight averaged to 69 kg. From October to December we have measured the weight and length of the fetuses, and this data was used to predict the calving date, all of which were in the period between the early April till late May. Fecundation and calving for the two-year-old hinds were more aligned, so they were all supposed to calve until April 20<sup>th</sup> of the following year, whereas for the three-year-old or older hinds the estimated time span for calving spread from the early April till late May. Sustainability of economy and survival of the population depend mostly on the success of the hinds during their lifetime.

(Keywords: red deer, *Cervus elaphus*, hind, body weight, conception/pregnancy, calving)

### INTRODUCTION

Number of red deer calves that a hind can biologically reproduce in a lifetime depends directly on the body weight of the hind. For hinds in red deer (*C. elaphus*) population the number of calves born by one hind ranges from 0 to 7 in a lifetime (Clutton-Brock *et al.*, 1982). There are several factors that influence the body weight of hinds, and therefore also their ability to be pregnant, the quality of the food sources in the area where the hinds live and the population density can be especially important (Duncan *et al.*, 1998). Considering the fact that the two sexes are living separate for most of the year, their social system is a matriarchate based on the family. As the number of the females (daughters) of the leading hind grows, so grows the total number of the herd. More head means higher population density and less sources of food, so a certain number of females do not reach the body weight necessary to start body processes that support reproduction (Brna, 1969). After successful breeding and growth and development of the fetus, calving occurs. The place of calving is carefully selected, because later on, during the first few weeks after calving, it is also the place where the young are kept. It is usually in high vegetation (grass, bushes), on dry ground and exposed to sun. Such care for the young is crucial for its survival during the first three months of its life

(Nikolandić and Degmečić, 2007). The mortality of the young can be caused by unfavourable weather conditions, small body weight after birth, poorer milk production of the hind, agricultural machines while mowing meadows and forest clearings as well as predators. A period of fast growth and development of the calves ensures them to be fit for winter. The entire reproduction cycle from breeding up to the year-old calf is very complex and the hind must gain experience in nursing the calf, so the results showing that the prime age hinds have the highest percentage of successful breeding (Langvatn et al., 2004). Young hinds are inexperienced in the tactics of hiding the young, whereas older hinds, who are not as fit, have poorer milk quality, so their young is underdeveloped comparing to the average ones, and such calves have a high mortality rate (Guinnes et al., 1978). In the nature even a small, insignificant advantage over others means survival and transference of such qualities and such small advantages to the future generations. This kind of selection is left up to nature, whereas we as breeders must secure all the necessary conditions in the habitat in order to maximize the production of calves and by selection process specifically exclude those hinds from the population that do not meet the required criteria for the success of each head.

## MATERIALS AND METHODS

Field data has been collected throughout the hunting season 2007/2008, on the area of the state hunting ground XIV/9 Podunavlje-Podravlje (Danube area, Croatia). In early November, more precisely November 6, 2007, during the hunting season 2007/2008 the first fetus has been found after a red deer hind (*C. elaphus*) has been culled. From that point on, field material has been used for this paper. The total number of culled hinds has been inspected (n=67 head). Out of 67 head, 48 head have been hinds, 3 years old or older, while the other 19 were two-year old females (yearling hinds). The following instruments were used for data collection: measuring tape with millimetre division (mm), weighing scale with decagram precision, weighing scale with gram precision. The following factors were measured (n=67): net weight – weight of the body in refrigeration plant (eviscerated game without head and lower legs), gross weight – calculation method (Clutton-Brock et al., 1982), pregnancy, number and sex of the fetuses (by inspection and counting), and body weight (weighing scale with gram precision) and length of the fetus (by placing the sewing thread onto the fetus from the top of its snout to the tip of its tail, following the spine, and transferring the thread onto the measuring tape and reading the length in millimetres) (Valentinčić, 1986). The data were processed with SPSS 16.0.

## RESULTS AND DISCUSSION

Managing wild game may aim to achieve production of healthy and high quality animals after they become mature in the economical sense, will be used as a product to be marketed, or the aim of the wild game management may be limiting or preventing damage caused by wild game in some other, currently more important, field of work (agriculture, forestry etc). Both aims, and especially the production, demand knowledge of the parameters of the population dynamics. This helps us understand the ways and conditions on which production resources are being renewed and improved in the nature. The population grows, stagnates or decreases depending on the achieved increase. This refers to the process that includes mating, calving, raising the young, the rate of total loss and results with the status before hunting starts. When discussing the stability of income

and sustainability of managing the red deer population hinds are extremely important as the bearers of production. Growth, stability or decrease in the population are determined by the achieved increase, and the increase may be improved if the number of calves increases that one hind in the population may give birth to (fecundity), if the rate of total loss is decreased or if the mating age for hinds is lowered (Silby and Hone, 2003). Many factors influence the body weight of hinds, and thus the ability to reproduce, but the quality of food sources in the area where the hind lives as well as population density must be especially emphasized (Clutton-Brock *et al.*, 1982; Mysterud *et al.*, 2002).

Out of 67 females, 50 were pregnant, that is 75% of successfully fecundated females. For yearling hinds the fecundation was successful in 47% of the cases, whereas for three-year old and older hinds fecundation was successful in 85% of the cases. The ways of life of the species from the deer population (red deer, roe-deer) that populate this area of Croatia as indigenous species in free nature, dictate the readiness for fecundation. A larger number of animals causes greater population density and decrease in food sources, so a certain number of females does not gain enough body weight to start pre-mating processes (Langvatn *et al.*, 2004).

The average body weight of the females that potentially could have mated is  $\Delta_{zuk}=64.57$  kg of net body weight, or 92.24 kg of gross body weight. According to the research of Clutton-Brock *et al.* (1982), the net body weight amounts from 60% to 73% of the gross body weight, depending on whether it is the males', fecundated females' or non-fecundated females' weight, and similar results have been found in the still unpublished research field material from eastern Slavonija (Spačva forest). Yearling hinds, took part in the mating process for the first time, had the average body weight of  $\Delta_{duk}=58.37$  kg net or 83.39 kg gross. The calculated average net body weight for fecundated yearling hind is  $\Delta_{dopl.}=65.88$  kg and for non-fecundated  $\Delta_{dnopl.}=51.60$  kg. By comparing the mean values of net body weight of the fecundated and non-fecundated yearling hinds it has been found that there is a significant difference between the net body weights of the specimens. The so called small specimen test "t-test" was used, the differences in mean values were distributed according to the Student "t" distribution ( $\Delta_{dopl.}=65.88$  kg,  $Sd^2_{dopl.}=71.73$ ,  $Sd_{dopl.}=8.47$ ;  $\Delta_{dnopl.}=51.60$  kg,  $Sd^2_{dnopl.}=126.04$ ,  $Sd_{dnopl.}=11.23$ ;  $t_{izr}=3.15$ , and is bigger than  $t_{0.05}=2.110$  and  $t_{0.01}=2.898$  – therefore there is a significant difference between the two groups).

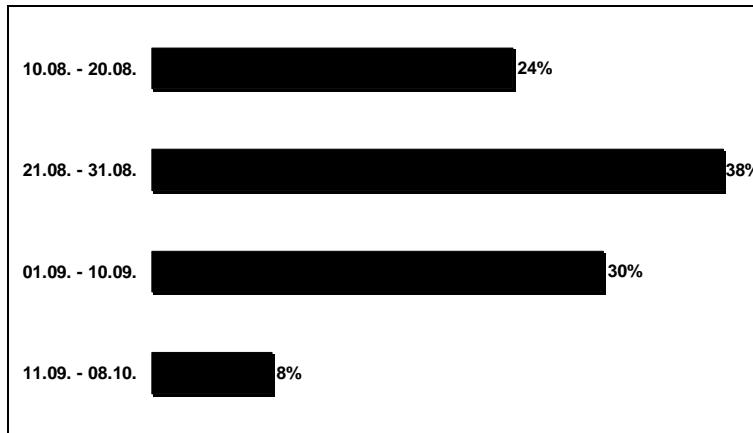
The average body weight of 3-year-old or older hinds is  $\Delta_{kuk.}=70.77$  kg net or 101.10 kg gross. Fecundated females weighed  $\Delta_{kopl.}=71.07$  kg, whereas non-fecundated females weighed  $\Delta_{knopl.}=69$  kg. The small specimen test (Student t-test) has shown that there are no significant differences between the average body weights of the two groups. Also, the mean net body weights of fecundated hinds by months of cull were calculated, and the results were:  $\Delta_{November}=68.92$  kg,  $\Delta_{December}=70.65$  kg,  $\Delta_{January}=67.73$  kg; there were no significant differences. Further, the mean values of body weight and length of the fetus by months of cull were calculated. According to Valentinčić (1991), the measuring of the fetus length determines the stage (week) of its development, which subsequently helps us to set the date of mating and the date of calving. By applying Valentinčić (1991) research results on our specimen we were able to calculate the average dates of fecundation and calving. The average dates of fecundation both in yearling hinds and in 3-year-old and older hinds are set in the last week of August, and if we add  $236.1\pm4.75$  days for male fetuses and  $234.2\pm5.04$  days for female fetuses (Clutton-Brock *et al.*, 1982), the calving date is set around April 25 the following year.

Figure 1 shows the dynamics of fecundation during 2007. The highest percentage of females, 38%, was fecundated in the period from August 21 to August 31, however

the period from August 10 to August 20 is no less important, since 24% of the females were fecundated at that time. The percentage in the first week of September was 30%, although it was expected to be higher. *Figure 2* shows the dynamics of the expected calving during 2008. It was expected that the most of the calves (38%) will be born around April 25, but the expected percentage of the so-called early calves to be born around April 15 is also important.

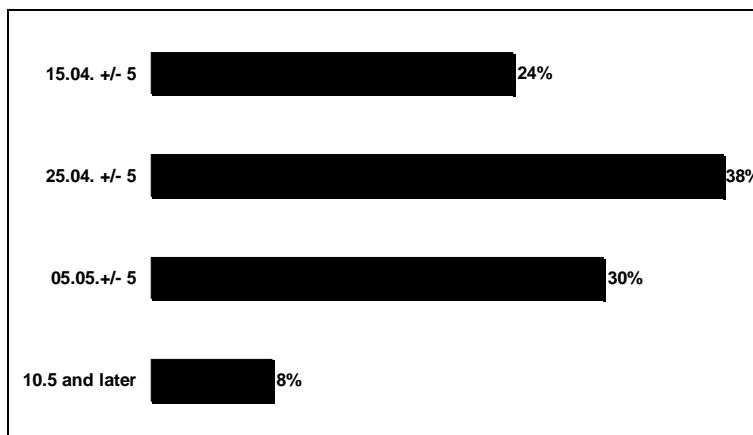
**Figure 1**

**The conception dynamics during Summer/Fall 2007**



**Figure 2**

**The dynamics of calving during Spring 2008**



Many factors influence the body weight of hinds, and thus the ability to reproduce, but the quality of food sources in the area where the hinds live as well as population density must be especially emphasized (Duncan et al., 1998). In his research on the fenced areas in Australia, Tuckwell (1998) found that 65 kg is the minimum gross body weight of red

deer females that are physiologically ready for mating, but he stresses that 80 kg is the desired gross body weight. Furthermore, he points out that the weight of 80 kg and more results in giving birth to more calves (which have larger body weights at calving), and who survive until their second year of life. The research mentioned in this paper confirms the body weight of females of about 80 kg as optimal for fecundation. Fecundated females that are 3 or more years old have the body weight of 70.77 kg net or 101.10 kg gross, whereas females that are going to mate for the first time, the so-called yearling hind, have the body weight of 65.88 kg net or 94 kg gross. The direct result of such body weights is the fact that 75% of females were successfully fecundated. The non-fecundated females that will mate for the first time have a significantly smaller body weight than the fecundated females, and it is 51.60 kg net or 73.71 kg gross, which confirms the fact that there is a certain threshold of body weight that enables mating and fecundation. Experts often point out the body fat index as a useful indicator of body fitness in wild ruminants (*Boen and Robinson*, 2003). In cases when hinds remain unfecundated that are 3 years old or older and meet the criterion of the needed body weight, they might be said to be barren, but still the fact has to be kept in mind that hinds may take a year's pause for resting in order to be fit for mating next year. It is also important to know that during one season, a hind is ready for fecundation (on heat) only two to three days and that this cycle is repeated every 18 days until she is fecundated or until the sex hormones are no longer secreted, which makes it possible that there was not a male deer around in those three days and so she could not have been fecundated (*Clutton-Brock et al.*, 1982). Apart from fecundation, the body weight in hinds is also important for the size and weight of the calf at the time of calving. Females who were calved as small calves, with body weight below the average of the management area, will also in the future, in all age groups, have under-average body weight and will produce weak calves who will not be able to catch up with their peers and contribute to the progress of the population management in a particular area (*Langvatn et al.*, 2004; *Clutton-Brock and Coulson*, 2003). Body size and body weight in females ensures that the calf will be bigger, and clearly, a stronger and bigger calf will sooner be able to follow the mother and escape from the predators (*Geist*, 1998).

The data on the length of the fetuses is used to draw conclusions on the dynamics of mating, on the process of fecundation and on the planned dynamics of calving. The data on the length of the fetuses in 2007 shows that the mating took place with different dynamics from approximately August 10 till approximately October 8. It is possible that some of the hinds conceived even later, but the field material did not provide proof that. The aim of game management is to place the mating period in as short a period of time as possible. The research in Scotland shows more than 70% synchronized conception in two weeks (*Clutton-Brock*, 1982). The results of the research conducted in the state hunting ground XIV/9 Podunavlje-Podravlje in 2007 show that 68 % of fecundation took place within around 20 days in late August and early September. A high percentage of early fecundation was observed in this area, from August 10 to August 20, as much as 24% of the specimen, and also a high percentage of fecundation from mid-September to mid-October, 8%. The explanation for early fecundation can be found in extremely favourable climate conditions which caused a rich and varied development of food base, whereas the cause of the high percentage of the so-called late fecundation can be found in a large number of two-year-old females and over aged hinds (old animals) who only later gained the body weight needed for fecundation or due to a lack of males ready for mating, so some hinds got fecundated in their third or fourth cycle. It is necessary to direct further research primarily on the age and sex structure of the population. It is clear

that a long mating period can only result in a long calving period. Early mating, before August 20, brought 24% of the early calved young who will display, and they were observed, above-average bodily development. Such calves can bring forth progress in the fecundity of females and in the trophy structure of the males. The late born young will have below average body weight due to the low body weight of the hinds, and will not have milk of the same quality as did the calves born in early spring, because the quality of nutrients in vegetation will decrease and so these calves will develop more slowly (Noyes et al., 2004; Brna, 1979). When body weight is met as a condition, the reproduction cycle and the beginning of the reproduction cycle is influenced by the photoperiod, weather conditions, adrenal gland and an intensive demonstration of sexual behaviour (scenting, characteristic cries and so on). Photoperiod or a response of the organism to a relative duration of lighter and darker periods ratio acts as a trigger for the reproduction cycle and stimulates the release of the adrenal hormones which trigger the activity of testicles and ovaries and thus the production of sex hormones. After the concentration of the sex hormones in blood increases, the animal displays sexual behaviour and the reproduction cycle begins. So, the amount of light or “duration of daylight” not only influences the physiological processes of the plants, but is also a trigger for all relevant physiological processes of the animals, as in the case of red deer (reproduction cycle, growth and development of the antler, moulting, lactation, and so on) (Lincoln, 1998).

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## The genetic diversity in two local Italian sheep breeds: is selective breeding against scrapie susceptibility possible?

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### **ABSTRACT**

*The genetic variability and presence of population substructures in two conserved native Northern Italian sheep breeds (118 samples), Alpagota and Brogna, not involved into scrapie eradication plans imposed by the European commission (2003), were studied by investigating 17 microsatellite markers. Obtained data were used for computer simulation of successive generations of the two breeds, according to a pattern of within breed crossings and to the choice of breeding males based on the genotypic data for scrapie susceptibility. Assumptions were made for the mating scheme: the allelic frequencies of the sampled individuals are representative of the original populations, all rams and females have the same reproductive performances, males are selected by random drawing and used only for one round of mating, the number of individuals making up the sample population is fixed. Four new subsequent generations were simulated by using two different approaches: discarding males with unfavorable scrapie genotype (Risk Class V) and without selection of the males. The total number of alleles detected in Alpagota was 158 (mean  $9.29 \pm 2.95$ ), and in Brogna was 186 (mean  $10.94 \pm 3.05$ ). Differences in the mean number of alleles, expected and observed heterozygosity, and molecular coancestry were not detected for the selected and unselected populations of both breeds. Results show that, if assumptions are met, the selection against scrapie sensitivity is possible in low diffusion local breeds without compromising genetic diversity.*

(Keywords: Alpagota, Brogna, sheep, scrapie, simulation)

### **INTRODUCTION**

Scrapie is a fatal neurodegenerative disease belonging to the group of transmissible spongiform encephalopathies, affecting sheep and goats. It has been shown that the susceptibility or resistance of sheep to scrapie is influenced mainly by mutation in the codons 136, 154 and 171 of the third exon of the prion protein (PrP) gene (*PRNP*) (Hunter, 1997). It is generally accepted that mutations coding for alanine at codon 136 (A<sub>136</sub>) and for arginine at codon 171 (R<sub>171</sub>) confer higher resistance, while those coding for valine (V<sub>136</sub>) and glutamine (Q<sub>171</sub>) render animals more susceptible to scrapie; polymorphisms in codon 154 are considered to have minor importance (Hunter, 1997; Elsen *et al.*, 1999). In the light of this scientific evidence, the European Union classified the genotypes at these three codons in five classes (Risk I: ARR/ARR, Risk II: ARR/AHQ AHQ/AHQ, Risk III: ARR/ARQ ARR/ARK ARR/ARH ARQ/ARQ ARQ/ARK ARQ/ARH ARQ/AHQ ARH/ARK AHQ/ARK AHQ/ARH, Risk IV:

ARR/VRQ ARK/VRQ AHQ/VRQ and Risk V: VRQ/VRQ ARQ/VRQ ARH/VRQ) from highly resistant (Risk I) to highly sensitive (Risk V) and established that breeding programmes aiming at decreasing scrapie susceptibility should be implemented in all European sheep breeds (EC, 2003). To achieve such goal breeding scheme must increase the frequency of the ARR/ARR genotype and eradicate completely the VRQ allele. However, application of these kind of schemes could be dangerous in many local breeds facing extinction as the available genetic variability will be reduced affecting conservation programmes as observed by several authors (Alfonso et al., 2006; Mann et al., 2007; Van Kaam et al., 2008). In Italy today there is a statutory exception that permits the exclusion from the plan for the selection of rare breed sheep entered in the herd book; is optional the admission of many breeders to plan in these cases. The analysis of genetic variability and the estimation of its possible loss could be assessed using different criteria mainly based on pedigree information, anyway in sheep breeding and especially in small local populations, pedigree information are often unknown (Goyache et al., 2003); in this case the use of molecular markers to evaluate molecular diversity could be a valuable alternative (Alfonso et al., 2006; Álvarez et al., 2007). The native breeds as Alpagota (ALP) and Brogna (BRO) analyzed, are historically tied to mountain communities of the Veneto Italian region and have always been used for the production of traditional dairy or meat products. The ALP is a small-sized sheep (Alpago mountains), and nowadays about 2.300 animals are enrolled in the herd book; it is listed in the FAO global databank for farm animal genetic resources where it is classified as endangered. It is a multipurpose breed (meat, milk, and wool), but now it is bred only for meat production due to its purported high quality. The BRO breed and is native to the Lessini mountains; at present the herd book accounts for about 1.450 animals. The BRO is a multipurpose breed (meat, milk, and wool), and its milk has been traditionally transformed to produce typical cheeses. The animals are medium to small in size and are very prolific, with a twinning rate of 58% as reported by *Pastore and Fabbris* (1999). The local sheep breeds as Alpagota (ALP) and Brogna (BRO), are reared in north-east Italy and nowadays they can be considered meat type. Their importance is due to their possibility to exploit marginal areas and to the production of typical products of economic interest in niche markets (es. "Alpago Lamb", Slow Food Presidia in the 2008). At present they are considered as endangered and are part of a conservation programme but no data on their susceptibility to scrapie are available.

Aim of this study was to simulate a mating scheme to evaluate the loss of heterozygosity in local sheep breeds under conservation scheme for reducing scrapie susceptibility.

## MATERIALS AND METHODS

In this study we have analyzed 118 animals, 23 males and 42 females for BRO and 27 males and 26 females for ALP. From each individual, 250 µl of blood was transferred on paper, FTA cards (FTA Nucleic Acid Collection, Storage and Purification, Whatman) for more rapid DNA extraction. Two disks obtained punched the FTA card were treated by running 3 washes of 5 minutes each in 200 µl of FTA Purification Reagent and then a wash of 5 minutes 200 µl TE Buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). The stage of purification was made by treating punches with 70% ethanol washes in cold running two centrifugal to 4000 RPM for 5 minutes. Once dry, the disks were used directly in the amplification mix. The extracted DNA was amplified by PCR at 17 microsatellite loci (*Table 1*). Two punches were added 15 µl total reaction mixture

containing 1X PCR buffer (16 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 67 mM Tris-HCl pH 8.8, 0.01% Tween 20), 0.35 µl of forward and reverse primer, 0.2 mM dNTPs, 0.3 mM of MgCl<sub>2</sub> and 0.01 U/µl of *Taq* DNA polymerase, in a final volume of 15 µl. Details on the primer annealing temperatures can be found in *Dalvit et al.* (2008). Allele size was determined with a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA). A panel of 17 microsatellite markers was chosen according to *ISAG/FAO Standing Committee* (2004) recommendations and to previous studies (*Baumung et al.*, 2006) to investigate highly polymorphic markers spread throughout the genome.

**Table 1**

**Microsatellite markers  
with corresponding fragment size and chromosomal location**

Locus	Fragment size	Ch.me
CSRD247	213–259	14
ILSTS87	138–178	6
OarCP34	100–128	3
OarFCB304	150–198	19
McM527	171–189	5
OarAE54	122–156	25
OarFCB20	92–122	2
URB058	161–209	13
OarAE129	137–157	5
OarAE119	147–185	19
INRA063	162–210	14
HSC	263–299	20
INRA023	196–224	1
MAF214	185–261	16
MAF65	121–143	15
OarCP49	69–119	17
TGLA53	140–168	12

Moreover, as a complete pedigree was absent, the investigated breeds were genotyped at 17 microsatellite loci to assess their genetic variability and to evaluate through simulations generations the possible variability loss if selection against sensitive genotypes will be carried out. The approach of simulation obtained by generating successive populations through Hybridlab software version 1.0 proposed by *Einar et al.* (2006), is used in this work to obtain information on temporal genetic populations of sheep which has available only a representative sample of the initial population but without adequate reference data. The following assumptions were made: a) the allelic frequencies of the sampled individuals are representative of the original populations, b) all rams and females have the same reproductive performances, c) males are selected by random drawing and used only for one round of mating, d) the number of individuals making up the sample population is fixed. For each real subject information about sex, scrapie genotype and the genotype at the 17 microsatellite loci and was used for selection and for the simulated mating process.

Population of 1000 samples was generated from the original individuals (real) (521 F and 479 M for ALP; 503 F and 479 M for BRO); these were considered all females was chosen as a number equal to 1/5 the number of females to males. By these males was extracted from a number equal to 1/20 (actual sex ratio in the populations investigated) the number of females from representative subgroups of allele frequencies (investigated for scrapie) real males. In this way it could create a generation simulated (pop\_T0) Representative actual frequency of males, consisting of adult females ( $F\alpha$ ), adult males ( $M\alpha$ ) and male comeback year for domestic ( $M\beta$ ). In the Figure 1 shows the pattern of intersections adopted. Composition in the column are shown the respective compositions of the populations placed under review, taking stock of the study at the end of each of the 4 hypothesized reproductive events after the selections and the lambs before the next breeding. 2 cases were postulated, the absence of selection (NO selection) and selection for scrapie eradication (YES selection). In the first case the males are sampled randomly from the entire gene pool of the offspring, while in the latter case, the sample of subjects from which to extract the animals for the comeback is reduced because it eliminates the class V. There is selection in females. The number of offspring equals the number of breeding females on average prolificacy of the breed (if 145% for ALP and BRO)

**Figure 1**

**Mating scheme used**

Time line (year)	Event	Population	Composition
0	$M\alpha + M\beta + F\alpha$		
0	$M\alpha \times F\alpha$	$\Rightarrow \text{pop\_T0}$	$M\alpha + M\beta + F\alpha$
1	$M\gamma + F\beta$		
1	$M\beta \times F\alpha$	$\Rightarrow \text{pop\_T1}$	$M\beta + F\alpha + M\gamma + F\beta$
2	$M\delta + F\gamma$		
2	$M\gamma \times (F\alpha + F\beta)$	$\Rightarrow \text{pop\_T2}$	$M\gamma + (F\alpha + F\beta)s + M\delta + F\gamma$
3	$M\epsilon + F\delta$		
3	$M\delta \times ((F\alpha + F\beta)s + F\gamma)$	$\Rightarrow \text{pop\_T3}$	$M\delta + ((F\alpha + F\beta)s + F\gamma)s + F\delta + M\epsilon$

M = males, F = females, + = and (no reproduction), x = mating (yes reproduction),  $\alpha$ - $\beta$ - $\gamma$ - $\delta$ - $\epsilon$  = progressive number generation, s = selection [ $F\alpha s = 90\% F\alpha$ ;  $F\beta s - F\gamma s - F\delta s = 10\% F\beta - F\gamma - F\delta$  (respectively);  $(F\alpha + F\beta)s = 90\% (F\alpha + F\beta)$ ;  $(F\alpha + F\beta)s + F\gamma s = 90\% (F\alpha + F\beta)s + F\gamma s$ ;  $M\gamma s - M\delta s - M\epsilon = 10\% (the number of F\alpha) of M\gamma - M\delta - M\epsilon$  (respectively)]

Number of alleles per locus, allelic frequencies, and observed and expected heterozygosity were calculated using Molkin version 3.0 and Genetix version 4.05.2

(Belkhir *et al.*, 1996-2004). A test for population differentiation was performed, as implemented in GENEPOP version 4.0. Molecular coancestry coefficients within and between breeds were measured according to Caballero and Toro (2002) using Molkin version 3.0 (Gutiérrez *et al.*, 2005). The differences for frequency scrapie genotype between observed and expected heterozygosity are tested by  $\chi^2$  test.

## RESULTS AND DISCUSSION

The total number of alleles detected in Alpagota (NO selection and YES selection) was 158 (mean 9.29, SD $\pm$ 2.95), and in Brogna (NO selection and YES selection) was 186 (mean 10.94, SD $\pm$ 3.05). The largest number of alleles was found at loci URB058 (16) for Alpagota (NO selection and YES selection) and OarCP49 (17) for Brogna (NO selection and YES selection); the smallest at locus McM527 (4) for Alpagota (NO selection and YES selection) and OarAE129 for Brogna (NO selection and YES selection). There is no difference between the number of alleles of the gene pool consists of 4 populations selected and the 4 selected for both Alpagota for Brogna. This indicates that if the assumptions were met would be possible to exclude the Risk Class V by selection without loss of genetic diversity. The genetic variability of each population was studied in terms of number of observed alleles and molecular coancestry, as shown in Table 2. The results of the analysis on simulated populations were consistent with those made by real people on Dalvit *et al.* (2009). The observed and expected heterozygosity were constant. Also the values of molecular coancestry constants are within-population. Considering then the percentages of allele frequencies (Table 3) is known as both slightly decreased the frequency of the VRQ allele in generations of populations subjected to selection for elimination of Risk Class V in both ALP and in BRO, while observing the opposite phenomenon in populations not subject to selection. Overall the differences for frequency scrapie genotype between observed and expected heterozygosity in all 4 populations was not significant ( $\chi^2$  test).

**Table 2**

**Number of analyzed sample (sample size), expected (H. exp.) and observed (H. obs.) heterozygosity, within-breed molecular coancestry (Kinsub) for each population analyzed**

Population	ALP_T0	ALP_T1	ALP_T2	ALP_T3	BRO_T0	BRO_T1	BRO_T2	BRO_T3	
Sample size	573	625	625	625	547	597	597	597	
NO selection	H. obs.	0.7555	0.7548	0.7541	0.7520	0.7894	0.7892	0.7846	0.7820
	SD	$\pm$ 0.1595	$\pm$ 0.1564	$\pm$ 0.1577	$\pm$ 0.1549	$\pm$ 0.105	$\pm$ 0.1005	$\pm$ 0.0964	$\pm$ 0.0975
	H. exp.	0.7385	0.7387	0.7392	0.7388	0.7640	0.7643	0.7656	0.7668
	SD	$\pm$ 0.1524	$\pm$ 0.1509	$\pm$ 0.1516	$\pm$ 0.1495	$\pm$ 0.088	$\pm$ 0.0883	$\pm$ 0.0873	$\pm$ 0.0868
	Kinsub	0.2615	0.2613	0.2608	0.2612	0.2360	0.2357	0.2344	0.2332
YES selection	H. obs.	0.7572	0.7523	0.7494	0.7443	0.7876	0.7851	0.7797	0.7783
	SD	$\pm$ 0.1586	$\pm$ 0.1587	$\pm$ 0.1594	$\pm$ 0.1576	$\pm$ 0.107	$\pm$ 0.1081	$\pm$ 0.105	$\pm$ 0.1041
	H. exp.	0.7385	0.7380	0.7384	0.7372	0.7639	0.7630	0.7627	0.7624
	SD	$\pm$ 0.1515	$\pm$ 0.1531	$\pm$ 0.1534	$\pm$ 0.1529	$\pm$ 0.0881	$\pm$ 0.0887	$\pm$ 0.0885	$\pm$ 0.0901
	Kinsub	0.2615	0.2620	0.2616	0.2628	0.2361	0.2370	0.2373	0.2376

**Table 3****Percentage of total alleles**

	Pop	Sample	% of total alleles					
			ARR	ARQ	AHQ	ARH	ARK	VRQ
NO selection	ALP_T0	1146	15.36	63.00	9.16	0.79	0.87	10.82
	ALP_T1	1250	14.88	64.16	8.88	0.64	0.88	10.56
	ALP_T2	1250	15.84	63.20	8.16	0.48	0.96	11.36
	ALP_T3	1250	15.92	63.36	7.92	0.40	1.04	11.36
	BRO_T0	1094	23.03	48.54	0.91	2.83	1.55	23.13
	BRO_T1	1194	23.28	48.07	0.84	2.93	1.68	23.20
	BRO_T2	1194	23.53	47.82	0.75	2.60	1.68	23.62
	BRO_T3	1194	22.95	48.66	0.75	2.18	1.68	23.79
YES selection	ALP_T0	1146	16.06	62.83	8.64	0.79	1.05	10.65
	ALP_T1	1250	16.56	62.80	8.72	0.72	0.96	10.24
	ALP_T2	1250	16.56	62.16	9.20	0.72	1.12	10.24
	ALP_T3	1250	16.40	61.92	9.60	0.96	1.28	9.84
	BRO_T0	1094	23.86	49.09	0.91	2.83	1.65	21.66
	BRO_T1	1194	23.37	49.75	0.84	3.35	1.76	20.94
	BRO_T2	1194	23.87	49.25	0.92	3.27	2.26	20.44
	BRO_T3	1194	24.54	49.83	0.84	3.10	1.93	19.77

**CONCLUSION**

In the Veneto local sheep breeds the selection plan for reducing scrapie susceptibility (EC, 2003) is not applied, according to Italian law which allows exemption of farm management. Moreover, the reduced number of the population is the problem that a possible selection as that prohibiting the use of sheep belonging to Risk Class V should gradually increasing the loss of heterozygosity in local sheep breeds. In this study it was possible to verify that the results of analysis on simulated populations were consistent with those made in previous study. The assumptions used in this study were upheld in reality, the plan coupled with selection and elimination of males belonging to the Risk Class V may be used, because:

- there is no difference between the number of alleles of the gene pool compost from 4 populations selected by the 4 selected for both Alpagota for Brogna,
- the molecular coancestry values are constant within-population,
- the simulation shows a decrease in the frequency of the VRQ allele in generations of populations subjected to selection, unlike those not subject to selection.

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## Growth performance and carcass traits of Croatian multicoloured breed kids

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### **ABSTRACT**

*The Croatian multicoloured goat is an autochthonous breed that belongs to a group of extensive Mediterranean breeds and represents the most of Croatian goat population. The aim of this paper was to evaluate growth potential, carcass traits and composition of male (36) and female (54) Croatian multicoloured breed kids. Kids were raised traditionally, on rangeland pasture from birth up to 24 kg live weight together with their mothers. Average birth weight of kids was 2.3 kg, daily gain 112 g, hot carcass weight 11.6 kg and dressing value 48.2%. Average percentage contribution to live weight of intestines was 31.5%, lungs and heart 1.99%, liver 1.86%, spleen 0.28%, testicles 0.78%, skin and feet 9.25% and horns 0.48%. Daily gain, dressing value, percentage contribution of some organs (spleen, skin and feet, horns) to live weight, were significantly affected by gender. Male goat kids had significantly longer hind limb and deeper chest than female kids. Despite their relatively high average age at slaughter and low growth rates, Croatian multicoloured goat kids had satisfactory dressing percentage and carcass characteristics.*

(Keywords: growth performance, goat kid, carcass composition, carcass measurements, sex)

### **INTRODUCTION**

The Croatian multicoloured goat is a very important kid meat producing animal in Croatia. It is an autochthonous breed that represents about 75% of the total goat population (HPA, 2009). The main breeding area of Croatian multicoloured goat are the coastal areas (Velebit and Dinara mountains, Dalmatian hinterland) where there is a lot of rocky ground and stone, poor vegetation, thicket and underbrush, and there are very few possibilities of breeding other types of stock (except sheep), especially larger stock. The body of mature Croatian multicoloured goats is medium sized (from 37 to 50 kg) and overgrown with thick, lengthy and dense hair of a black-white, brown or grey-white colour and rarely of a single colour. The head is medium sized, with obligatory horns mostly curving backwards, shaped like a sabre (Mioč *et al.*, 2008). It has not been registered that this breed was significantly and systematically improved in any period, except some sporadic attempts. Hence, we can acknowledge that it was originally created in this area and that it belongs to a group of extensive Mediterranean breeds (Rako *et al.*, 1979).

The main breeding goal of Croatian multicoloured breed is kid meat production, while milk secretion lasts until weaning or slaughtering of offspring. Despite its importance, Croatian multicoloured goat has received little attention. Also, to our knowledge, there is no published information relating to the previously mentioned goat

breed kids' growth and carcass traits. The objective of the present study was to establish growth performance, carcass traits and composition of male and female Croatian multicoloured breed kids.

## MATERIALS AND METHODS

A total of 90 single born Croatian multicoloured breed kids (36 males and 54 females) were used for this experiment. Kids were raised traditionally, on rangeland pasture from birth up to 24 kg live weight together with their mothers. In order to get accurate records for birth weights, all investigated kids were weighed within 12 hours of birth. Kids were weighed and slaughtered after an overnight fast at a local slaughterhouse. Daily gain of kids was calculated as a difference between slaughter weight and birth weight, divided by days of age at slaughter. The dressed carcass comprised body after removing the skin, fore feet (at the carpal-metacarpal joint), hind feet (at the tarsal-metatarsal joint), and the viscera. Kidneys, kidney and pelvic fat were retained in carcass, and testes and scrotal fat were also removed (according *Colomer-Rocher et al.*, 1987). Hot carcass weight and weights of the skin and feet and some visceral organs (heart, lungs plus trachea, liver, spleen) were recorded. The gastro-intestinal tract was weighed full. The results were expressed as percentage of live weight at slaughter. Dressing percentage was calculated based on full live weight. Carcass measurements included, carcass length: from the caudal edge of the last sacral vertebra to the dorso-cranial edge of the atlas vertebra; hind leg length: from the centre of the tuberosity on the proximal end of the tibia to the distal edge of the tarsus; chest depth: the greatest depth, measured in a horizontal plane on the hanging carcass; width of buttock: the greatest width, measured in a horizontal plane on the hanging carcass (*Fisher and de Boer*, 1994) and chest width: the greatest width, measured with callipers in a horizontal plane on the hanging carcass. Data were analyzed by using MEANS procedure, and variance analysis was performed using the GLM procedure (*SAS*, 1999) by using the model which included fixed effect of sex and random residual error. Least square means were computed and tested for differences by the Tukey-Kramer test.

## RESULTS AND DISCUSSION

Mean birth weight of kids in our research was similar to earlier study on Croatian multicoloured breed (*Babić*, 1940). The birth weight of a kid depends primarily on the conformation and size of the adults of the breed to which it belongs (*Morand-Fehr*, 1981). Thus, Croatian multicoloured breed kids (*Table 1*) had considerably lower mean birth weight than some European dairy breed kids (*Majid et al.*, 1993), like Saanen and Toggenburg kids (3.8 and 3.5 kg, respectively).

Also, mature size of the sire and dam is one of the major influences of kids' growth. Hence, average growth rate of 112 g/day in the present study was lower than average growth rates attained for Saanen crossbred kids of similar age (140–167 g/day) in a study by *Dhanda et al.* (2003). Despite relatively low average daily gain, Croatian multicoloured kids were heavier than weaned kids of larger breeds at the same age, such as Anglo-Nubian and Saanen (*Dickson et al.*, 1990). This is in accordance with results of the previous studies, which demonstrated that greater growth rates of goat kids in the pre-weaning period are followed by a post-weaning depression of growth rates (*Palma and Galina*, 1995).

In the present study, kids' dressing percentage (48.2%) was higher than compared to Boer crossbred goats and Canary Caprine Group breed of similar live weight at slaughter (Ryan *et al.*, 2007; Marichal *et al.*, 2003). According to the data presented in *Table 1*, average percentage contribution to live weight of intestines was 31.5%, lungs and heart 1.99%, liver 1.86%, spleen 0.28%, testicles 0.78%, skin and feet 9.25% and horns 0.48%.

**Table 1**

**Descriptive statistics for slaughter data of Croatian multicoloured-breed kids**

	$\bar{x}$	Sd	$S_{\bar{x}}$	min	max	CV, %
Birth weight (kg)	2.3	0.64	0.07	1.0	3.5	27.8
Daily gain (g)	112.0	27.0	2.7	73.0	241.0	24.1
Age (d)	189.3	32.2	3.3	103.0	315.0	17.0
Live weight (kg)	24.1	3.1	0.3	18.0	33.5	12.8
Hot carcass weight (kg)	11.6	1.5	0.2	8.2	16.6	13.1
Dressing percentage (%)	48.2	2.2	0.23	40.6	52.5	4.5
Intestines (kg)	7.6	1.2	0.1	5.5	10.9	15.5
Lungs and heart (g)	480.0	88.6	9.3	301.0	795.0	18.5
Liver (g)	449.5	62.8	6.6	310.0	658.0	14.0
Spleen (g)	68.6	19.3	2.06	34.0	114.0	28.2
Testicles (g)	189.1	66.4	11.3	63.0	327.0	35.1
Skin and feet (kg)	2.23	0.26	0.03	1.73	2.93	11.9
Horns (g)	116.7	52.2	7.1	42.0	236.0	44.7

Carcass measurements of Croatian multicoloured kids are presented in *Table 2*. Ekiz *et al.* (2010) reported significant influence of breed on carcass measurements. Dhanda *et al.* (1999) reported higher length for larger size genotypes (e.g. Boer or Saanen crosses) compared to Angora or Feral crosses. In comparison with kids in our study, Turkish Saanen suckling kids with average slaughter weight of 13.3 kg, had higher hind limb length and buttock width (28.35 and 13.43 cm, respectively).

**Table 2**

**Descriptive statistics for carcass measurements (cm)**

Carcass measurements	$\bar{x}$	Sd	$S_{\bar{x}}$	min	max	CV, %
Carcass length	61.17	3.14	0.33	52.00	67.50	5.13
Hind limb length	25.02	1.17	0.12	20.60	27.50	4.68
Chest depth	23.72	1.10	0.11	21.50	26.50	4.64
Chest width	11.51	0.72	0.07	9.80	13.50	6.29
Buttock width	12.95	1.37	0.15	10.80	23.80	10.58

Birth weights did not differ between sexes (*Table 3*), which is not in agreement with results of Morand-Fehr (1981) who reported significantly higher birth weights of male kids compared to females for several goat breeds.

**Table 3****Least square means ( $\pm$ SE) of male and female kids' performance, carcass traits and non-carcass components**

Traits	Male (n=36)	Female (n=54)	P value
Birth weight (kg)	2.33 $\pm$ 0.09	2.25 $\pm$ 0.09	0.56
Daily gain (g)	121.7 $\pm$ 0.01	110.4 $\pm$ 0.01	<0.05
Age (d)	184.9 $\pm$ 4.8	193.9 $\pm$ 4.6	0.17
Live weight (kg)	24.8 $\pm$ 0.5	23.7 $\pm$ 0.4	0.08
Hot carcass weight (kg)	11.8 $\pm$ 0.3	11.5 $\pm$ 0.2	0.49
Dressing percentage (%)	47.3 $\pm$ 0.3	48.8 $\pm$ 0.3	<0.01
Intestines (%)	31.9 $\pm$ 0.6	31.3 $\pm$ 0.5	0.39
Lungs and heart (%)	2.01 $\pm$ 0.05	1.98 $\pm$ 0.04	0.65
Liver (%)	1.85 $\pm$ 0.04	1.89 $\pm$ 0.03	0.46
Spleen (%)	0.26 $\pm$ 0.01	0.30 $\pm$ 0.01	<0.01
Skin and feet (%)	9.7 $\pm$ 0.2	9.1 $\pm$ 0.2	<0.05
Horns (%)	0.66 $\pm$ 0.02	0.32 $\pm$ 0.02	<0.001

According to other studies (Singh et al., 2009), male Croatian multicoloured kids had higher average daily gain ( $P<0.05$ ) and higher live weight at slaughter ( $P<0.08$ ) than female kids (Table 3). Furthermore, male kids had significantly ( $P<0.01$ ) lower dressing value than females. Similarly, Živković and Knežević (1991) reported lower dressing percentage in male kids from Croatian multicoloured  $\times$  Alpine crossbred genotypes compared to females, although the difference was not significant. Significant difference between sexes in our study for dressing percentage based on live weight was mainly attributed to the variations in the weight of spleen, skin and feet and horns at slaughter. However, it was difficult to make comparisons with most of the literature because of the different genotypes, rearing systems, age and slaughter weight, etc. used.

Male goat kids had longer hind limb ( $P<0.05$ ) and deeper chest ( $P<0.01$ ) than female kids (Table 4), which may be due to higher live weight of male kids at slaughter. On the contrary, Santos et al. (2007) and Peña et al. (2007) found significant difference between sexes only for carcass length in Serrana and Florida kids, respectively. However, Peña et al. (2007) reported an increase in carcass measurements with increasing slaughter weight.

**Table 4****Least square means ( $\pm$ SE) of male and female kids' carcass measurements (cm)**

Carcass measurements	Male (n=36)	Female (n=54)	P value
Carcass length	61.63 $\pm$ 0.52	60.87 $\pm$ 0.43	0.26
Hind limb length	25.38 $\pm$ 0.19	24.78 $\pm$ 0.15	<0.05
Chest depth	24.14 $\pm$ 0.18	23.44 $\pm$ 0.14	<0.01
Chest width	11.49 $\pm$ 0.12	11.52 $\pm$ 0.10	0.84
Buttock width	13.18 $\pm$ 0.23	12.80 $\pm$ 0.19	0.20

## CONCLUSIONS

This initial study provides the basis for estimating non-genetic effects of sources of variation of growth performance and carcass traits and allows a well-documented evaluation of this indigenous Croatian goat breed not yet selected. Despite their relatively high average age at slaughter and low growth rates, Croatian multicoloured goat kids had satisfactory dressing percentage and carcass characteristics. In order to accomplish better economic efficiency of this breed in existent production system, further researches on evaluation of meat production potential of this breed under controlled management are necessary.

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## Body composition of crossbred kids evaluated by Computed Tomography

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### ABSTRACT

*Sixteen crossbred female kids, (Hungarian Milking Brown×Alpine) F<sub>1</sub> (n=8) and (Hungarian Milking Brown×Boer) F<sub>1</sub> (n=8), were selected from the same farm. The kids were scanned by Computed Tomography to evaluate and compare the body composition of crossbred progenies. The area of fat, muscle and bone tissues were estimated according to the x-ray densities from the 10 mm thick scans. The distance were 20 mm between slices, therefore forty-sixty slices were produced by individual, depending on the longness of vertebral column. The area of tissues were recorded in mm<sup>2</sup> and analysed by GLM using live weight as covariant (LSD-test; P<0.05) and partial correlation corrected for body weight. In Alpine crossbreds the average area of fat tissue was 64.6 thousand mm<sup>2</sup>, while in Boer progenies it was 71.1 thousand mm<sup>2</sup>. The area of muscle tissue in Alpine crossbred kids was 290.4 thousand and 372.0 thousand mm<sup>2</sup> in Boer crossbreds, which difference was significant. The average area of bone tissue was similar in two crossbreds groups. In Alpine F<sub>1</sub> none of the partial correlation among fat, muscle and bone tissues became significant. In Boer F<sub>1</sub> the correlation between muscle and bone tissues was strong and significant. The Boer had stronger and significant effect on body tissue composition comparing to Alpine breed concerning to meat production.*

(Keywords: kid, body composition, tissue, computed tomography)

### INTRODUCTION

The image creating diagnostic methods (like ultrasonography, x-ray computed tomography, MRI) are wide spread used to estimate the quantity of meat produced by animals. The computed tomography method is based on the x-ray absorption of different tissues. The most important advantage of CT is that the animals can be scanned as frequently as it is necessary.

The veterinarians and animal scientists have used the CT methods in studying almost all animal species (poultry, rabbit, porcine, fish, sheep and bovine) in the University of Kaposvár (Hungary) over the last twenty years.

In goat species, limited information is available concerning the results of „in vivo” methods. *Junkuszew and Ringdorfer* (2005) studied lambs by ultrasonography and CT as well, to estimate the tissue composition and compare the two methods. They found the CT more accurate in prediction, but the ultrasonography was cheaper. *Delfa et al.* (1996, 1998, 1999) studied the relationships between the ultrasound measurements in live adult goats and the measurements of fat thickness and muscle depth taken on carcass. According to *Toldi* (2003) the S/EUROP body conformation of lambs could be predicted

from CT data with medium correctness ( $r=0.68$ ), while the relationship was strong in the case of fat cover ( $r=0.87$ ), and the strongest value ( $r=0.94$ ) was observed in the case of hot carcass weight.

The objective of this study was to evaluate and compare the body composition of Alpine and Boer firstcross kids.

## MATERIALS AND METHODS

Sixteen female kids were selected randomly from the same farm. The eight (Hungarian Milking Brown×Alpine)  $F_1$  kids were six months old, while the other eight kids belonging to (Hungarian Milking Brown×Boer)  $F_1$  had 4 months of age.

The animals were starved 12 hours and transported to the Health Sciences Centre of the University of Kaposvár. In the preparation room, the kids were weighted with 0.5 kg accuracy. Two-three kids were parallel placed in an examination hutch and were drug in muscle with 0.3–0.4 mg/body weight Xilazin fifteen minutes before scanning. If necessary further 0.1 mg/body weight drug was injected in, and they were placed in ventral position in the plastic examination trough. The front legs were placed near head while rear legs were locked caudale position. CT scans were performed by Siemens Somatom Expert Plus 4 equipment. In all segments the area of fat, muscle and bone tissue were measured and recorded. Scan pictures series were made by the “Australian method” (Mezőszentgyörgyi, 2000) with a 10 mm slice thickness and 20 mm slice distance. The number of pictures (40–60) taken was depended on the longness of vertebral column.

The pictures were evaluated by Medical Image Processing V1.0 software (Závoda, 2006). Fat, muscle and bone tissues were recorded in  $\text{mm}^2$  by CTPC programme based on the Hounsfield Units (1980) (*Table 1*). Microsoft Excel macros were made to process the data matrix.

**Table 1**

### The Hounsfield Units of body tissues

Type of tissues	Hounsfield Units
fat	from -200 to -20
muscle	from +20 to +200
bone	from +600 to +1000

The individual data received were analysed using SPSS 10.0 software. Means, standard deviations and GLM (Generalized Linear Model) using live weight as a covariant (LSD-test;  $P<0.05$ ) procedure were conducted to compare the two genotypes. The relationships among tissue areas corrected for body weight could be shown using partial correlation.

## RESULTS AND DISCUSSION

The average body weight of kids at the time of CT examination were lower in Boer crossbreds (16.9 kg) than in the case of Alpine progenies (17.9), but this difference were not significant (*Table 2*).

In Alpine  $F_1$  the average area of fat tissue was 64.6 thousand  $\text{mm}^2$ , while in Boer  $F_1$  it was 60.9 thousand  $\text{mm}^2$  – the observed difference was not significant. The most

important muscle tissue in Alpine crossbred kids was 290.4 thousand mm<sup>2</sup>, while in Boer F<sub>1</sub> kids 302.5 thousands mm<sup>2</sup> were found. The measured 12.9 thousands mm<sup>2</sup> difference in muscle tissue was also not significant. The average area of bone tissue was similar in two crossbreds groups, 53.8 thousand mm<sup>2</sup> in Alpine and 44.6 thousand mm<sup>2</sup> in Boer crossbred kids. The muscle/fat ratio was 25% bigger in Boer F1 kids than in Alpine crossbreds, however the differences were not significant (P=0.158), probably due to the low number of measured kids (*Table 2*).

**Table 2**

**Average (±standard deviation) body weight (kg), average values of fat, muscle and bone tissues (1000 mm<sup>2</sup>)**

	<b>Body weight</b>	<b>Fat tissue</b>	<b>Muscular tissue</b>	<b>Bone tissue</b>	<b>Muscle/Fat ratio</b>
(HMB×Alpine) F <sub>1</sub>	17.88 ±2.42	64.60 ±20.57	290.44 ±42.73 <sup>a</sup>	53.77 ±13.18	4.73 0.98
(HMB×Boer) F <sub>1</sub>	16.94 ±5.45	60.92 ±39.94	302.53 ±119.78 <sup>b</sup>	44.60 ±19.27	5.89 1.98

<sup>a,b</sup> P≤0.05

In Alpine crossbred kids there was low negative correlation between fat and bone tissue. The correlations among fat, muscle and bone tissues were low and none of the correlations became significant. In the case of Boer crossbred kids the correlations were stronger (medium and strong) than in Alpine crossbreds. The correlation between muscle and bone tissue was found strong and significant (*Table 3*).

**Table 3**

**Partial correlation coefficients among different tissue areas  
(corrected for body weight)**

	<b>Fat tissue</b>	<b>Muscle tissue</b>	<b>Bone tissue</b>
Fat tissue		0.37	-0.25
Muscle tissue	0.67		0.29
Bone tissue	0.53	0.83*	

\* P≤0.05; Values above diagonal are for (HMB×Alpine) F<sub>1</sub> kids; under diagonal for (HMB×Boer) F<sub>1</sub> kids

## **CONCLUSIONS**

However, the Boer crossbred kids were younger than Alpine progenies at the time of examination, and had lower body weight, they had higher area of all measured tissues, particularly the most important muscle tissue. It meant that the Boer goat had stronger and significant effect on body tissue composition comparing to Alpine breed concerning to meat production.

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## The daily yield, the physico-chemical characteristics of goat milk during lactation

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### **ABSTRACT**

*Average milk yield and milk composition (fat, protein, lactose) and their changes over the lactation were studied on 156 Hungarian Milking White, 168 Hungarian Milking Brown, 106 Hungarian Milking Multicolour goats from April to October, at 4-weeks interval. Daily milk yield and milk composition were counted from the morning and evening yield and contents. Does of Hungarian Milking Multicolour started the lactation with the highest daily yield, while from the second part of the lactation, the Hungarian Milking White does had the highest average daily yield. In the course of lactation the Hungarian Milking Brown had the lowest daily yield. The fat content of the milk increased from 3.2% to 5.0%, with the highest fluctuation in Hungarian Milking Brown producing the highest fat content at the beginning and at the end of lactation. The protein content of the milk increased from 3.1 to 3.9%. In Hungarian Milking White the protein content increased persistently, however, in Hungarian Milking Brown and Hungarian Milking Multicolour the value of this quality decreased from the second period, and reincreased from the 111<sup>st</sup> day. During the milking period the lactose content of milk changed between 4.3 and 4.6%. The highest lactose content was measured in Hungarian Milking White, while the lowest in Hungarian Milking Multicolour milk.*

(Keywords: goat, milk, fat, protein, lactose)

### **INTRODUCTION**

The goats are widely used for milk production in European countries, while in Africa the meat production has important rule in human consumption. In the developed countries the lactation curves of different goat breeds and the chemical composition of milk during lactation were already analysed.

According the results of *Mellado et al.* (1991) the goats reached the peak yield at third month in the first, third and fourth lactation, while the second lactation peak yield was produced in the fourth month after kidding. The daily yield increased until 5–8<sup>th</sup> week, then decreased continuously until the end of lactation (*Schandl*, 1966). *Bodó* (1959) results showed a steady milk production and a decrease from the third-fourth month. *Claps et al.* (2007) analysed four Mediterranean goat breeds (Maltese, Red Syrian, Ionica, Gingentana) concerning chemical composition of the milk, and showed a significantly higher fat and protein content produced by Maltese than other breeds. According to *Sollberger and Schäeren* (2003) results brienz goats' milk contained more fat and protein compared to Saanen breed. *Katanos et al.* (2005) measured similar fat content in Saanen like *Bedő et al.*

(1999). Sung et al. (1999) compared Alpine, Nubian, Saanen and Toggenburg goat milk, where fat percentages of Alpine and Toggenburg were medium and that of Nubian was higher than the total average. Protein percentage of Nubian was also higher than in the other three breeds and that of Alpine was the lowest. According to the result of Mimosi et al. (2007) the fat, protein and lactose content increased during lactation. The Hungarian local goats produced less protein (3,4%) content than Alpine (3,5%) and Saanen (3,5%) (Csapóné et al., 2009). The objective was to estimate the lactation curve of the Hungarian local goats, and to study the changes in fat, protein and lactose contents over the lactation.

**Table 1****Milk production of Hungarian goats**

Breed	Lactation milk yield (litre)	Daily milk yield (litre)	Source
Hungarian Milking White	177.4	1.3	Pintér et al., 2004
Hungarian Milking White	303.6	1.8	Kukovics, 2005 <sup>1</sup>
Hungarian Milking White	287.2	1.7	Kukovics, 2005 <sup>2</sup>
Hungarian Milking Brown	242.2	1.7	Pintér et al., 2004
Hungarian Milking Brown	268.3	1.7	Kukovics, 2005 <sup>1</sup>
Hungarian Milking Brown	314.4	2.0	Kukovics, 2005 <sup>2</sup>
Hungarian Milking Multicolour	222.9	1.5	Pintér et al., 2004
Hungarian Milking Multicolour	278.6	1.8	Kukovics, 2005 <sup>1</sup>
Hungarian Milking Multicolour	285.9	1.8	Kukovics, 2005 <sup>2</sup>

<sup>1</sup>data from 2003, <sup>2</sup>data from 2004**Table 2****Fat, protein and lactose content of goat milk**

Breed	Fat (%)	Protein (%)	Lactose (%)	Source
Saanen	3.90	3.40	4.70	Bedő et al., 1999
Red Syrian	3.47	3.34		Claps et al., 2007
Girgentana	3.97	3.13		Claps et al., 2007
Ionica	4.30	3.66		Claps et al., 2007
Maltese	4.70	3.91		Claps et al., 2007
Damascus	5.06	3.25	4.64	Katanos et al., 2005
Saanen	3.82	3.14	4.51	Katanos et al., 2005
Saanen×Alpine	3.88	3.14	4.66	Katanos et al., 2005
Saanen×local Greek	4.79	3.56	4.73	Katanos et al., 2005
(Saanen×local Greek)×Saanen	3.95	3.47	4.63	Katanos et al., 2005
Payoya	4.91	3.73		Mena et al., 2007 <sup>1</sup>
Payoya	4.25	3.54		Mena et al., 2007 <sup>2</sup>
Payoya	4.53	3.68		Mena et al., 2007 <sup>3</sup>
Payoya	5.53	4.09		Mena et al., 2007 <sup>4</sup>
Camosciata	3.52	3.40		Mimosi et al., 2007 <sup>5</sup>
Camosciata	3.45	3.34		Mimosi et al., 2007 <sup>6</sup>
Saanen	3.07	2.69		Sollberger and Schäeren, 2003
Oberhalsi-Brienz	3.31	2.82		Sollberger and Schäeren, 2003
Alpine	3.40	3.08	4.37	Sung et al., 1999
Nubian	4.48	4.23	4.16	Sung et al., 1999
Saanen	2.55	3.25	4.56	Sung et al., 1999
Toggenburg	3.54	3.21	4.16	Sung et al., 1999

<sup>1</sup>Jan., March; <sup>2</sup>Apr., June; <sup>3</sup>July-Sept; <sup>4</sup>Oct-Dec; <sup>5</sup>early lactation; <sup>6</sup>end of lactation

## MATERIALS AND METHODS

Five goat herds, three small and two bigger farms were including in a test day milk recording program. Does were belonging to 3 breeds, like Hungarian Milking White (HMW, n=156), Hungarian Milking Brown (HMB; n=168), Hungarian Milking Multicolour (HMM, n=106). Individual milk yield was measured and individual milk samples were taken twice a day, morning and evening, at 4-weeks intervals from April to October. The length of lactation was separated into 7 intervals (51–80<sup>th</sup>, 81–110<sup>th</sup>, 111–140<sup>th</sup>, 141–170<sup>th</sup>, 171–200<sup>th</sup>, 201–230<sup>th</sup>, 231–260<sup>th</sup> days after kidding). The physico-chemical properties of milk were determined by reference and standard methods, by an official raw milk laboratory (Livestock Performance Testing Ltd. in Gödöllő, Hungary). The fat, protein and lactose content (%) were measured by Milkoscan 600 instrument. The morning and evening yields were summarized. The daily fat, protein and lactose content were counted as follows:

$$\text{daily fat \%} = \frac{(\text{MY} \cdot \text{MF}) + (\text{EY} \cdot \text{EF})}{\text{MY} + \text{EY}} \quad (1)$$

$$\text{daily protein \%} = \frac{(\text{MY} \cdot \text{MP}) + (\text{EY} \cdot \text{EP})}{\text{MY} + \text{EY}} \quad (2)$$

$$\text{daily lactose \%} = \frac{(\text{MY} \cdot \text{ML}) + (\text{EY} \cdot \text{EL})}{\text{MY} + \text{EY}} \quad (3)$$

Where:

MY=morning yield (litre);

EY=evening yield (litre);

MF=morning fat%;

EF=evening fat%;

MP= morning protein%;

EP=evening protein%;

ML=morning lactose%;

EL=evening lactose%.

## RESULTS AND DISCUSSION

The does of Hungarian Milking Multicolour started the lactation with the highest daily yield, while from the second part of the lactation, the Hungarian Milking White does had the highest average daily yield. In the course of lactation the Hungarian Milking Brown had the lowest daily yield. The Hungarian Milking White and the Hungarian Milking Multicolour does had the peak daily yield (1.6 litre) in the period of 81–110 days, while the Hungarian Milking Brown the peak daily yield (1.5 litre) was in the period of 111–140 days. At the beginning of the milking season the daily milk yield was between 1.4 and 1.5 litres, but in the second period the yield increased to 1.5 and 1.6 litre in all three breeds. The yield stayed at the same level in the Hungarian Milking White and the Hungarian Milking Multicolour breeds, and from the period of 171–200 days the yield decreased to 1.0 litre at the end of lactation. The milk production of the Hungarian Milking Brown decreased after the peak yield to 0.9 litres. During lactation, in the period of 171–200<sup>th</sup> days, there was significant difference ( $P \leq 0.05$ ) found between Hungarian Milking White and Hungarian Milking Brown (*Figure 1*).

The fat content of the milk increased from 3.2% to 5.0%, with the highest fluctuation in Hungarian Milking Brown producing the highest fat content at the beginning and at the end of lactation. The Hungarian Milking Multicolour does produced 3.4% milk fat until the 170<sup>th</sup> day, then it strongly increased. The tendencies were similar in other two breeds, however, the fat content increased from 3.3 to 3.5% in Hungarian Milking White, then it was stable until the 200<sup>th</sup> day, and then intensive increase was observed. Significant differences ( $P \leq 0.05$ ) were found in the period of 111–140<sup>th</sup> days between Hungarian Milking White and Hungarian Milking Brown (Figure 2).

The protein content of the milk increased from 3.1 to 3.9%. In Hungarian Milking White its value increased persistently, however, in Hungarian Milking Brown and Hungarian Milking Multicolour the protein content decreased from the second period, and reincreased from the 111<sup>st</sup> day. In the course of the lactation the differences among breeds changed, until the 140<sup>th</sup> day the protein yield was the highest in Hungarian Milking Multicolour does, but in the further period, in the Hungarian Milking Brown breed. The differences between breeds were not significant during lactation (Figure 3).

During the milking period the lactose content of milk changed between 4.3 and 4.6%. The highest lactose content was measured in Hungarian Milking White, while the lowest in the milk of Hungarian Milking Multicolour does, which differences were significant in almost all, except the periods of 81–110 days. In the two last periods of lactation the lactose content of milk produced by Hungarian Milking White and Hungarian Milking Brown does were significantly different (Figure 4).

**Figure 1**

**Daily milk yield during lactation**

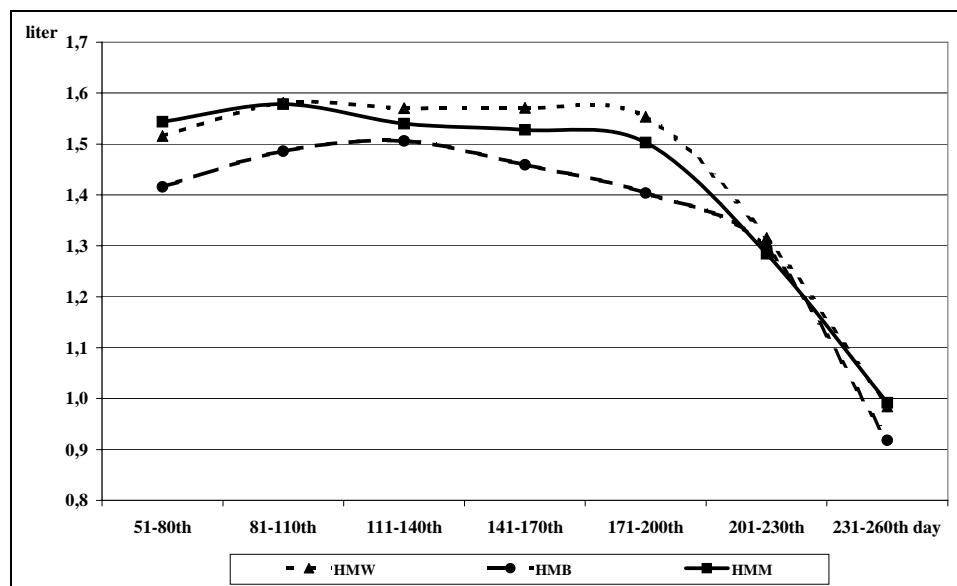


Figure 2

**Daily fat content during lactation**

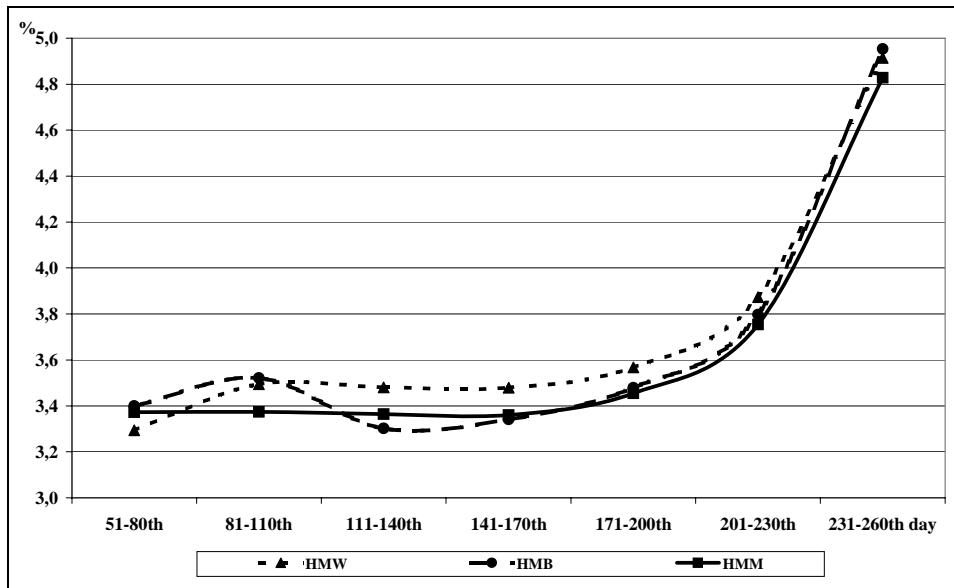
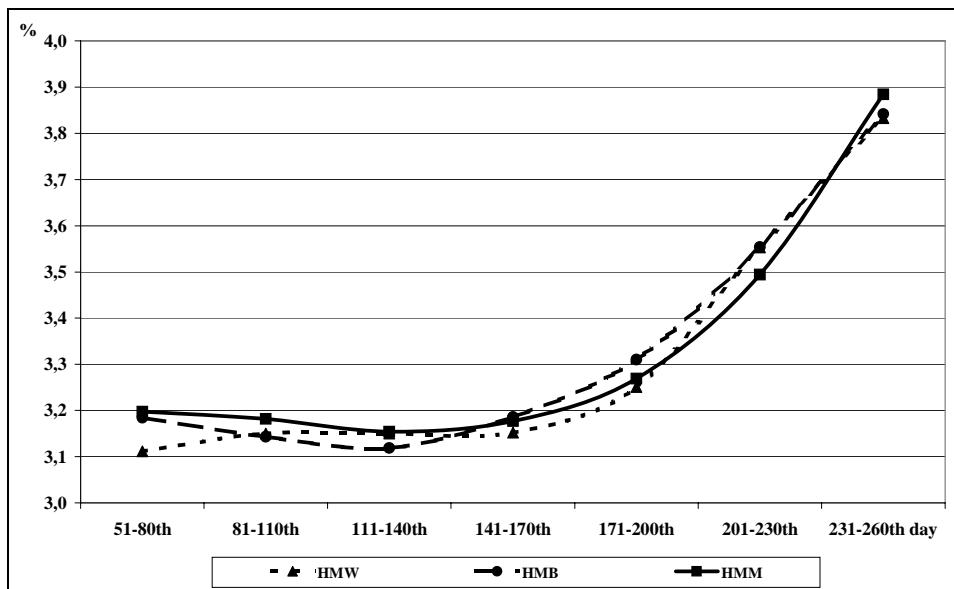
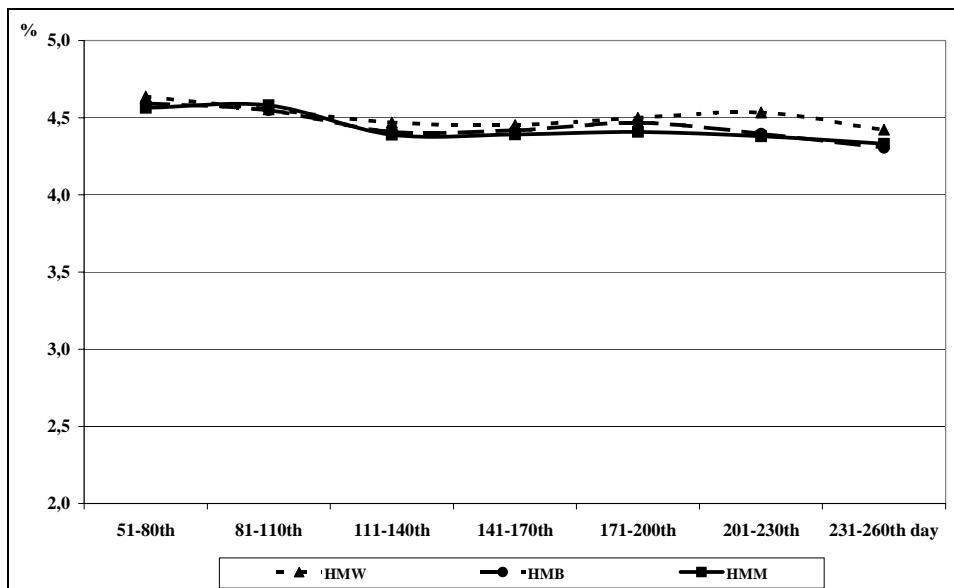


Figure 3

**Daily protein content during lactation**



**Figure 4****Daily lactose content during lactation****CONCLUSIONS**

The average daily yield (1.4 litre) of Hungarian Milking White does were higher than published by Pintér et al. (2004), but lower than the results of Kukovics (2005). In the Hungarian Milking Brown and Hungarian Milking Multicolour breeds Pintér et al. (2004), and Kukovics (2005) showed higher daily yield than 1.3 and 1.4 litres.

According to our results the fat content of milk changed between 3.2 and 5.0%, like in the study of Mimosi et al. (2007). Mena et al. (2007) also showed an important increase in fat content from April to December. Kukovics et al. (2009) and Katanos et al. (2005) published similar fat content to our results (3.7%) in all three Hungarian breeds.

The changes in protein content (from 3.1 to 3.9%) during lactation did agree with the results of Mena et al. (2007). The average daily protein content during lactation (3.3%) was similar to Claps et al. (2007) in Red Syrian, Mimosi et al. (2007) in Camosciata, Bedő et al. (1999) in Saanen breeds.

The changes in lactose content (between 4.3 and 4.6%) were in average 4.4–4.5% for the whole lactation like the data published by Katanos et al. (2005).

It could be proposed to start the milking period as soon as possible after the kidding, and finish or starting the dry season after 200 days, because of high decrease of milk yield even if the fat and protein content increases.

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## Effect of different ratio of rapeseed cake in feed on production of hens and fatty acids content of egg yolks

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### ABSTRACT

*The main goal of this research was to establish the influence of 8% (PN-8) and 16% (PN-16) rapeseed cake in feed mixture on production of hens and fatty acids content of eggs yolk. 90 hens of Hrvatica strain have been used in period of 24<sup>th</sup> to 32<sup>th</sup> weeks of egg production. Hens have been sorted out in 30 cages, 3 of them in each cage. Hens from group PN-16 laid significantly ( $P<0.05$ ) lighter eggs and had highest consumption of feed mixture/kg of eggs. The nutrition treatment had not clearly negative effect on average egg production during the investigation. The mortality of 10% in group PN-16 and 6.67% in group PN-8, points to the possible negative impact of rapeseed cakes on the health of hens. Feeding hens the diet with rapeseed cake decreased ( $P<0.05$ ) SFA, increased PUFA n-3 and PUFA n-6 content of egg yolks and had positive effect ( $P<0.05$ ) on PUFA n-6/PUFA n-3 ratio. Taking into consideration above mentioned, it can be concluded that the rapeseed cake can be recommended to be used in the portion of 8 % in feed mixture for Hrvatica laying hens.*

(Keywords: rape seed oil-cake, laying hens, production, fatty acid, eggs)

### INTRODUCTION

Rapeseed cake is a good source of crude protein, but the limiting factors in poultry nutrition is a high content of crude fibre and antinutritive components: glucosinolates, eruca acid and tannin (Chibowska *et al.*, 2000). Campbell *et al.* (2007) estimated that feeding hens with rapeseed cake did not affect the egg production and mortality of hens. Feeding hens the diet with ground rapeseed increased PUFA n-3 and PUFA n-6 content of egg yolks, and had positive effect on PUFA n-6:PUFA n-3 ratio (Niemiec *et al.*, 2002). The main goal of this research was to establish the influence of 8% (PN-8) and 16% (PN-16) rapeseed cake in feed mixtures on production of Hrvatica layers and fatty acids content of their egg yolks.

### MATERIALS AND METHODS

While conducting the experiment, 90 hens of Hrvatica strain, have been used, in period of 24<sup>th</sup> to 32<sup>th</sup> weeks of egg production. Breeding of Hrvatica hen started at the beginning of 20<sup>th</sup> century and today is rearing in four strains (Janječić *et al.*, 2007). Hens have been sorted out in 30 cages, 3 of them in each cage. Ratio of rapeseed cake (rape seed cultivar Bristol) in feed mixtures was 0% (PN-0), 8% (PN-8) and 16% (PN-16). Each nutrition treatment had ten replicate. Chemical compositions of rapeseed cake and feed mixtures for hens are shown in *Table 1*.

**Table 1****Chemical composition of rapeseed cake and feed mixtures for hens**

Chemical composition, %	Rapeseed cake	PN-0	PN-8	PN-16
Moisture	6.48	11.70	11.26	11.30
Ash	6.93	9.23	10.48	10.46
Protein	30.31	16.94	16.66	16.96
Fat	7.70	5.18	6.01	6.11
Fibre	12.60	3.60	4.15	4.34
Nitrogen free extracts	35.98	53.35	51.44	51.10
Ca	1.02	2.31	2.66	2.55
P	1.27	0.75	0.67	0.65

During the investigation the average egg weight, average egg production, conversion of feed mixtures and mortality of hens were measured. Content of fatty acid of 30 samples of eggs yolk (10 of each treatment) were analysed. The dried lipid extract was methylated according to Hartman and Lago (1973). Fatty acid methyl esters were separated on a gas chromatograph (Philips, PU 4550) equipped with a split injector (100:1), fused silica capillary column (50m×0.25 mm i.d., 0.20 µm film thickness of polyethylene glycol (CP-SIL 88, Crompak, Netherlands), flame ionisation detector, and work station (Borwin, France). The fatty acids were identified by comparison of the retention times of the sample with those of the standards, and by co-chromatography. To verify the differences between the eggs yolk, the results for fatty acids were submitted to an analysis of variance (ANOVA) at the 5% level of confidence (SAS, 2007).

**RESULTS AND DISCUSSION**

Production results of hens of Hrvatica strain are given in *Table 2*.

**Table 2****Production results of Hrvatica layers during eight weeks of eksperiment**

Production result	Treatment		
	PN-0	PN-8	PN-16
Average egg mass g	57.02 <sup>a</sup>	57.34 <sup>a</sup>	56.90 <sup>a</sup>
Average egg production of hens, %	45.50	49.92	44.03
Consumption of feed mixture/ kg of egg mass	3.93 <sup>b</sup>	3.96 <sup>b</sup>	4.61 <sup>a</sup>
Mortality, %	0	6.67	10

<sup>a,b</sup> P<0.05

Hens from group PN-16 laid significantly lighter (P<0.05) eggs (P<0.05) and had highest consumption of feed mixture/kg of eggs. The nutrition treatment had not clearly negative effect on average egg production during the investigation regarding mortality. The mortality of 10% in group PN-16 and 6.67% in group PN-8, points out the possible negative impact of rapeseed cakes on the health of hens. Fatty acid composition (grouped as SFA-saturated fatty acids, MUFA- mono-unsaturated fatty acids, PUFA poly-unsaturated fatty acids) of rapeseed cake, layer feed mixture and egg yolks are given in *Table 3*.

**Table 3**

**Grouped fatty acid composition (% of total) of rapeseed cake,  
layer feed mixtures and egg yolks**

Fatty acids	Rapeseed cake	Layers feed mixture			Eggs yolk		
		PN-0	PN-8	PN-16	PN-0	PN-8	PN-16
ΣSFA	13.27	26.44	25.52	25.49	35.03 <sup>a</sup>	33.46 <sup>b</sup>	33.72 <sup>b</sup>
ΣMUFA	54.82	28.91	30.3	30.21	48.43	48.79	47.75
Σ PUFA n3	6.66	1.84	2.66	2.71	1.04	1.35	1.48
Σ PUFA n6	22.62	37.04	35.46	35.58	13.87	14.79	15.51
ΣSFA/ΣMUFA	0.24	0.91	0.84	0.84	0.72	0.69	0.71
Σ PUFA n6/Σ PUFA n3	3.40	20.13	13.33	13.13	13.34 <sup>a</sup>	10.96 <sup>b</sup>	10.48 <sup>b</sup>

<sup>a,b</sup> P<0.05

Feeding hens the diet with rapeseed cake decreased (P<0.05) SFA, increased PUFA n-3 and PUFA n-6 content of egg yolks and had positive decreasing-effect (P<0.05) on PUFA n-6/PUFA n-3 ratio. This could be valid because the ratio of n-6/n-3 PUFA in human food should be as much as possible to approximately 1 (*Okuyama et al.*, 1997).

## CONCLUSIONS

Taking into consideration above mentioned, it can be concluded that the rape seed oil-cake can be recommended to be used in the portion of 8% in feed mixtures for Hrvatica laying hens.

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## Development of new image evaluation software and its applicability in the in vivo prediction of egg yolk content in hen's eggs depending on some CT aquisition parameters

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### ABSTRACT

*The present study was designed to determine the in vivo predictability of egg yolk content in hens' eggs by means of computer tomography (CT), depending on some aquisition parameters. The experiment was carried out with altogether 120 eggs, which were originated from a 36 week old TETRA-H parent stock. During the CT measurements eggs were positioned in egg holders (10 eggs), thus two eggs were scanned simultaneously. The scanning parameters were: 80–110–130 kV and 40–80–120 mAs in 9 possible combinations, spiral mode, pitch 1, field of view 110 mm. In all cases eggs were scanned using overlapping 3 mm slice thickness on a Siemens Somatom Emotion 6 multislice CT scanner. On the images obtained the volume of the yolk was determined using a self-developed egg-separation and segmentation software. After the CT measurements eggs were broken and their yolk weight was measured. Pearson correlations were calculated between the CT predicted yolk volume and the measured yolk weight. It was established that the higher tube voltage settings of 110 and 130 kV resulted in higher correlation ( $r=0.78-0.79$ ) between these two examined traits than the lower voltage setting of 80 kV (0.75–0.76). The X-ray dose (mAs) had no significant effect on the correlation coefficients. Based on these results it was concluded that further development of this method is needed in order to obtain the similar accuracy of prediction in the case of egg components as it was already reached in the case of body composition in different animal species. For this purpose the egg-segmentation software has to be tested with modified algorythms. Further optimization of the measurement parameters might need to be considered as well.*

(Keywords: hen, egg, yolk content, computer tomography)

### INTRODUCTION

Over a long period of time, elucidation of the correlations between the composition of hatching eggs and the development of the birds hatched was hampered by the lack of instruments that would have been capable of determining the composition of eggs and monitoring the development of embryo within the same egg without opening the egg. Later, *Williams et al.* (1997) made an attempt to determine the chemical composition of eggs without opening them, using the so-called TOBEC method for their study. During their studies conducted with chicken, duck, guinea fowl and quail eggs, they demonstrated a significant positive correlation between the so-called E values measured

by the TOBEC method (the electrical conductivity of the eggs) and the water content of eggs as well as the dry matter content of the albumen in all the four species studied. However, between the E-value and the dry matter content of the egg yolk statistically significant correlations were found only in the case of chicken and quail eggs.

Relying on the results of *Williams et al.* (1997), studies on the composition of hen's eggs using the TOBEC method, without opening the eggs, together with the investigation of correlations between egg composition, hatchability and the development of the birds hatched have recently been started at the Kaposvár University as well. The study demonstrated that, using the TOBEC method – by measuring the electrical conductivity – eggs with a composition markedly different from one another can be distinguished and assorted efficiently. At the same time, the research results have confirmed that eggs of different composition – i.e., having dissimilar yolk/albumen ratios – have significantly deviating hatchability, and that the birds hatching from these eggs have significantly different body composition at the time of hatching and significantly different growth rate during rearing and finishing (*Milisits et al.*, 2008a, 2008b). Thus, these research results clearly show that the composition of hatching eggs markedly influences their hatchability and, furthermore, it has an impact on the quality of the birds hatched and even on their growth rate during the rearing and finishing period. On the basis of all these findings, therefore, it seems to be expedient and justifiable to continue the research in order to determine the correlations between egg composition, hatchability and the development of the birds hatched in a more accurate manner. Namely, the biggest disadvantage of the above-mentioned TOBEC method is that, because of the only moderate correlation found between the electrical conductivity and the composition of eggs, it is not suitable for demonstrating minor changes in egg composition, and is reliable only for distinguishing eggs with extremely divergent composition (*Milisits et al.*, 2007). Therefore, in a former experiment, the applicability of computer tomography (CT) was tested for the *in vivo* prediction of the composition of hen's eggs.

However, in that study it was pointed out that the evaluation of the CT images based on the X-ray density values of the pixels (picture elements) resulted only in a very low correlation between the measured yolk weight and the estimated yolk volume (*Milisits et al.*, 2009). Therefore, as another method of the evaluation, the surface of the egg yolk was determined manually on the cross-sectional CT images, which resulted in a much better accuracy of prediction, depending on the number of scans involved in the prediction equations (*Milisits et al.*, 2009). However, because this evaluation method is very time-consuming, a new egg-separation and segmentation software was developed for the automatic determination of egg yolk volume on the CT images obtained. Therefore, the aim of this study was to examine, whether the accuracy of prediction of the egg yolk content can be influenced by changing the technical parameters of the CT scanning procedure.

## MATERIALS AND METHODS

As first step of the study, CT examination of 120 eggs – originating from a dual-purpose TETRA-H parent stock – was carried out in order to develop the measurement protocol and to determine the correlations between the information content of the CT images and yolk content of the eggs. The CT examinations were performed by a Siemens Somatom Emotion 6 multislice CT scanner, based at the Institute of Diagnostic Imaging and Radiation Oncology of the Faculty of Animal Science at Kaposvár University.

Before the scanning procedure eggs were weighed and positioned for the scanning in standing/upright position. During the CT measurements eggs were positioned in egg

holders (10 eggs), thus two eggs were scanned simultaneously. Altogether 9 scanning settings were tested, where the tube voltage and the X-ray radiation dose were 80 kV–40 mAs, 80 kV–80 mAs, 80 kV–120 mAs, 110 kV–40 mAs, 110 kV–80 mAs, 110 kV–120 mAs, 130 kV–40 mAs, 130 kV–80 mAs, 130 kV–120 mAs. At all of these settings 3 mm thick overlapping slices were taken from the eggs using the following technical parameters: spiral mode, pitch 1, field of view 110 mm. The images obtained were analysed by a new self-developed egg-separation and segmentation software. With the help of this software the border of the shell and albumen and the border of the albumen and yolk was determined and the volume of the yolk was calculated thereafter.

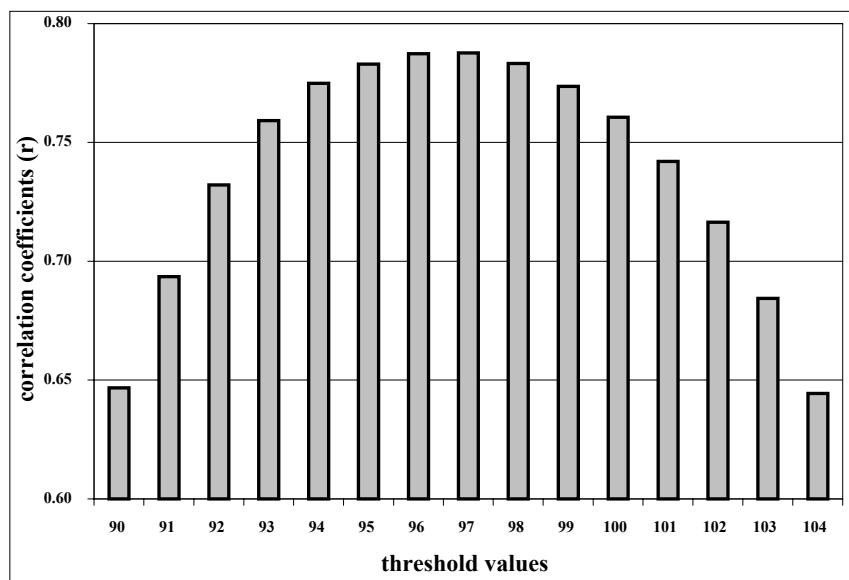
After the CT measurements all of the eggs were broken and their yolk and albumen were separated. After weighing the yolk, its ratio to the whole eggs was calculated. For the determination of correlations between CT predicted yolk volume and measured yolk weight Pearson correlation coefficients were calculated using the SPSS statistical software package (*SPSS for Windows*, 1999).

## RESULTS AND DISCUSSION

Because the newly developed egg-separation and segmentation software is based on finding the border between shell and albumen and albumen and yolk, the first step of the evaluation was the determination of the strongest correlation between predicted and measured egg yolk content depending on the applied threshold values for separating the different egg components. As a result of this evaluation it was found that the use of the value 97 resulted in the most accurate separation of albumen and yolk, i.e. in the highest correlation between the predicted and measured yolk content of the eggs (*Figure 1*).

**Figure 1**

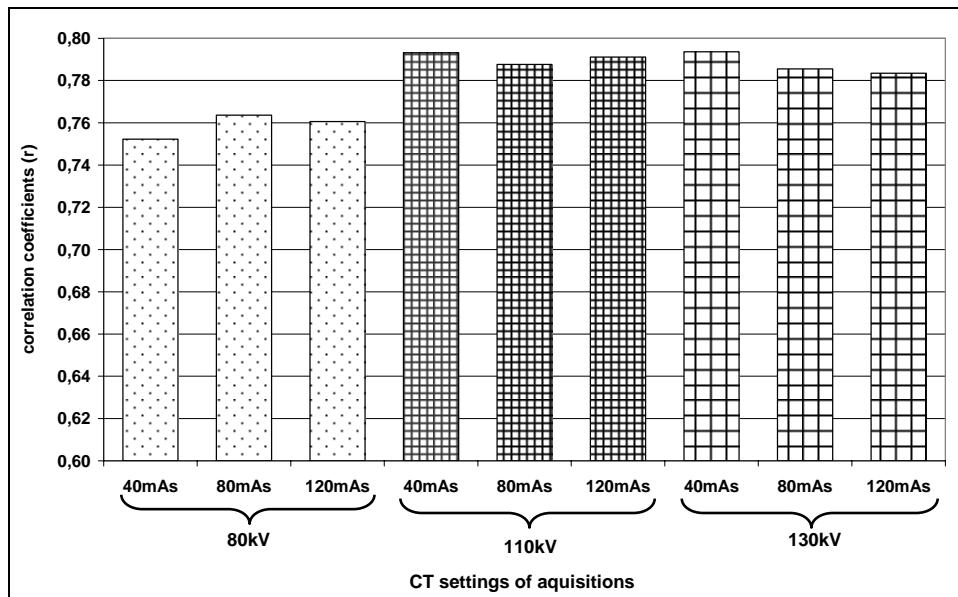
**Correlations between CT predicted and weighed egg yolk content of hen's eggs using different threshold values in the image evaluation**



Therefore, the further evaluation of the effect of different measurement parameters on the predictability of the egg yolk content was based on using this threshold value. Using this semiautomatic evaluation process it was established that the higher tube voltage settings of 110 and 130 kV resulted in higher correlation coefficients ( $r=0.78-0.79$ ), than the lower voltage setting of 80 kV which had only an  $0.75-0.76$  “ $r$ ” value between the CT predicted yolk volume and the measured yolk weight. The X-ray dose (mAs) had no significant effect on the examined correlation coefficients (Figure 2).

**Figure 2**

**Correlations between the CT predicted yolk volume and the measured yolk weight depending of the CT acquisition parameters**



## CONCLUSIONS

Based on the results it was concluded that the obtained correlation coefficients were lower than it was expected, but they were better than in a former experiment using the TOBEC method. It is likely, that further modification of the aquisition parameters is needed using more scans – smaller slice thickness – covering the eggs during the measurement procedure. The other possible way of the development could be the adaptation of new algorithms into the egg-separation and segmentation software, which could increase the correlation between the estimated and measured egg yolk content.

## ACKNOWLEDGEMENT

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## Computer tomograph study of changes in the body composition of dual-purpose chicken genotypes between 4 and 12 weeks of age

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### ABSTRACT

*The aim of this study was to compare the changes in the body composition of Tetra-H chicks, reciprocal crossbred progenies and chicks from a new cock line, which is planned to use as a new parental line in the breeding program of the TETRA-H. Altogether 90 chicks (15 in both sexes in all genotypes) were involved into the experiment and were scanned biweekly by means of a SIEMENS Somatom Emotion 6 multislice CT scanner between 4 and 12 weeks of age. During the scanning procedure a total of 20 scans were made of each animal, using 8 mm slice thickness and different distances between the scans, depending on the length of the vertebrae. Using this method, scans with the same serial number represent the body composition at the same anatomical points, and so animals of different sizes could be compared. Based on the results it was established that the highest liveweight was reached by the animals of the new cock line at the end of the experiment. However, the lowest ratio of the muscle and the highest ratio of the fat was also observed in these animals, which means an unfavourable body composition at the slaughter compared to the other two genotypes. Therefore, it was concluded that the use of the new cock line as new parental line in the breeding program of the TETRA-H seems to be good for increasing the liveweight of the TETRA-H chicks, but it could be unfavourable for the body composition.*

(Keywords: chicken, body composition, computer tomography)

### INTRODUCTION

The three-line hybrid TETRA-H was developed in the beginning of the 1980's by the Bábolna Poultry Breeding Company. Thanks for its calm temperament and balanced production it was capable of replacing the traditional free-range poultry breeds. However, out of the dual-purpose characteristics, the egg-production of these birds is the dominant one (180–200 pieces under extensive circumstances) and therefore the increase of their body weight seems to be needed by keeping the good growing ability in the early weeks of the rearing. Concerning the meat-production ability of the new genotype the theoretical average body-weight would be between 2000 and 2400 g at 8–9 weeks of age as a target parameter. However, with increasing the final body weight, some unfavourable changes could be happen in the body composition of the animals and therefore the examination of changes in the body composition of the new cock line seems to be needed before using it in the breeding program. Therefore, the aim of this study was to compare the changes in the body composition of TETRA-H chicks, reciprocal crossbred progenies and chicks from a new cock line during the growing

period by means of computer tomography (CT), which was already effectively used in a lot of former experiments in the *in vivo* examination of changes in the body composition of different species (Romvári et al., 1998; Milisits et al., 1999; Milisits et al., 2000; Andrásy-Baka et al., 2003).

## MATERIALS AND METHODS

The experiment was carried out with TETRA-H chicks, reciprocal crossbred progenies and chicks from a new cock line in the Test Station of the Kaposvár University, Faculty of Animal Science. Animals were reared on deep litter in pens (9.2 m<sup>2</sup> basic area), in a closed building, separated according to sex and genotype (cocks: 110 birds/pen, pullets: 129 birds/pen). Chicken were fed *ad libitum* with commercial diets during the whole experimental period (starter between days 0 and 10, growing between days 11 and 24 and finisher from the 25<sup>th</sup> day on (*Table 1*). Drinking water was also continuously available from self-drinkers.

**Table 1**

### Composition of the diets used in the experiment

Component	Starter	Growing	Finisher
Dry matter (%)	91.4	91.3	90.0
Crude protein (%)	20.9	18.8	17.1
Crude fat (%)	5.7	6.5	6.8
Crude fibre (%)	2.4	2.7	3.0
Crude ash (%)	4.9	4.4	4.4
N-free extract (%)	57.5	58.9	58.7
Starch (%)	39.2	48.5	48.5
ME Poultry (MJ/kg dry matter)	13.64	15.54	15.27
Calcium (g/kg)	7.34	6.11	6.47
Phosphorous (g/kg)	5.70	5.80	5.40

Animals for the CT examinations – 15 according to sex in all of the three genotypes – were chosen randomly at 4 weeks of age. These birds were then assigned individually with wing tags and they were scanned by CT at every examination days thereafter. Before the CT measurements the liveweight of these birds was always recorded.

CT examinations were taken at the Institute of Diagnostic Imaging and Radiation Oncology of the Kaposvár University bi-weekly, between 4 and 12 weeks of age. During the measuring procedures birds were fixed with belts in a special plexi-glass container, without using any anaesthetics. Three animals were scanned simultaneously.

The CT measurements consisted of overlapping 8 mm thick slices covering the whole body using a Siemens Somatom Emotion 6 multislice CT scanner. Using the images obtained the muscle and fat indices were calculated by determining the ratio of number of pixels with X-ray density values of muscle or fat with the total number of pixels with density values of muscle, water and fat, i.e. the range between -200 to +200 on the Hounsfield-scale:

$$\text{Muscle index} = \Sigma(+20)-(+200) / \Sigma(-200)-(+200) \cdot 100 \quad (1)$$

$$\text{Fat index} = \Sigma(-200)-(-20) / \Sigma(-200)-(+200) \cdot 100 \quad (2)$$

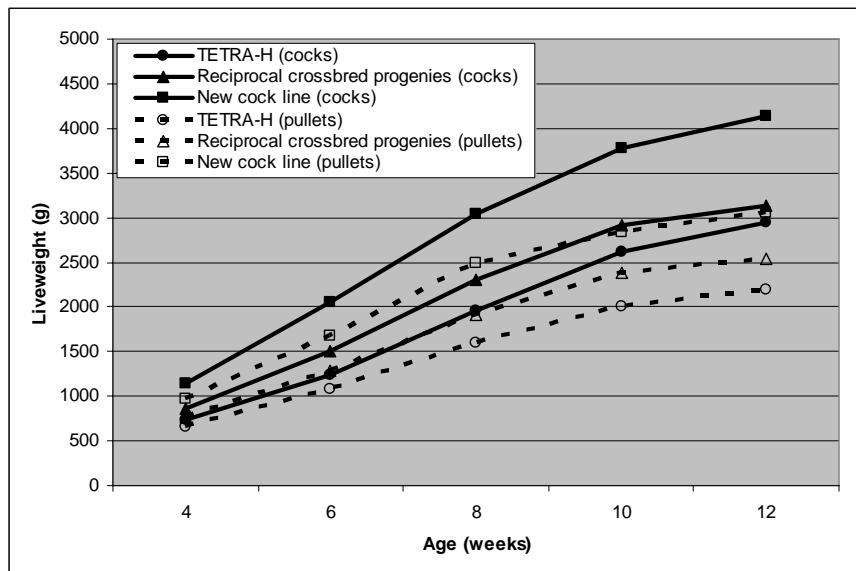
The differences in the liveweight and in the muscle and fat indices between the examined genotypes were evaluated statistically by the One-Way ANOVA method. The significance of the between group differences was tested by the LSD post hoc test. The statistical analysis was carried out by the SPSS statistical software package, version 10.0 (*SPSS for Windows*, 1999).

## RESULTS AND DISCUSSIONS

Examining the changes in the liveweight of the chicks it was established that the liveweight of the three genotypes differs significantly ( $P<0.05$ ) from each other in both sexes at all examined ages (*Figure 1*).

**Figure 1**

### Changes in the liveweight of TETRA-H chicks, reciprocal crossbred progenies and chicks from the new cock line between 4 and 12 weeks of age

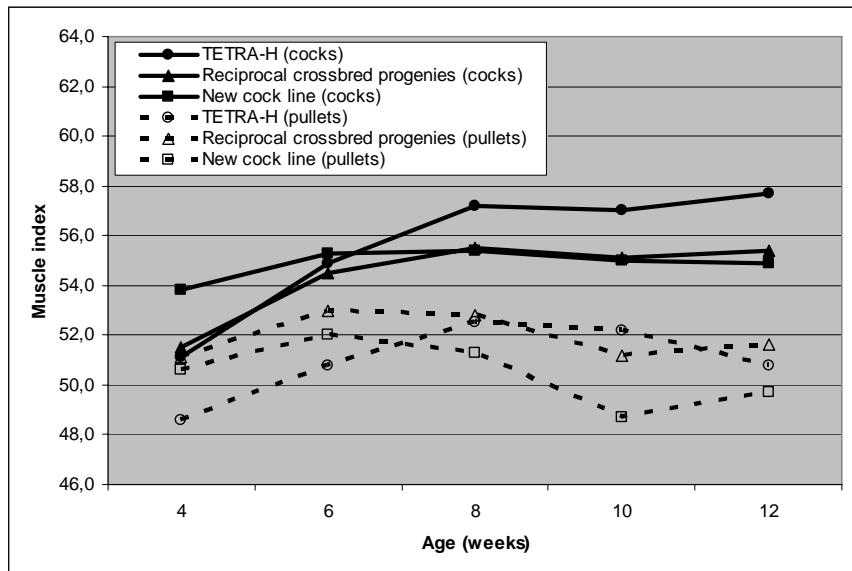


The highest liveweight was reached by the animals of the new cock line, while the lowest by the TETRA-H hybrids at the end of the experiment. The superiority of the new cock line was more than 1 kg in the case of cocks and almost 900 g in the case of pullets at 12 weeks of age.

The liveweight was permanently increasing in all of the experimental groups till the 10<sup>th</sup> week of age, except the pullets of the new cock line. In the last two weeks of the experiment a little break was observed in the increasing of the liveweight in both sexes in all of the three genotypes.

It was interesting to see that the liveweight of the pullets of the new cock line was higher at the end of the experiment, than that of the TETRA-H cocks (3064 g vs. 2950 g).

In the case of the muscle index an increase was observed in all of the three examined genotypes and in both sexes between 4 and 6 weeks of age (*Figure 2*).

**Figure 2****Changes in the muscle index of TETRA-H chicks, reciprocal crossbred progenies and chicks from the new cock line between 4 and 12 weeks of age**

However, from that time forth the muscle index remained at this level in the cocks of the new cock line till end of the experiment, while it was increasing till 8 weeks of age in the case of cocks of the other two genotypes. At 4 weeks of age the muscle index of the cocks from the new cock line was significantly ( $P<0.05$ ) higher than that of the cocks of the other two genotypes, but at the end of experiment they reached the lowest value, which significantly ( $P<0.05$ ) differed from that of the TETRA-H cocks. The highest ratio of the muscle in the body was observed in TETRA-H cocks at 12 weeks of age, which was higher by 4.2% and 5.1% than that of the other two genotypes.

In the case of the pullets the muscle index decreased from 6 weeks of age in the case of the reciprocal crossbred progenies and the new cock line genotypes, while it was increasing till 8 weeks of age in the TETRA-H genotype. At 12 weeks of age the worst result was produced by the birds of the new cock line also in this case, but the difference from the other two genotypes was not statistically significant ( $P>0.05$ ) in this case. The fat indices were decreasing between 4 and 6 weeks of age in both sexes and in all of the examined genotypes (Figure 3).

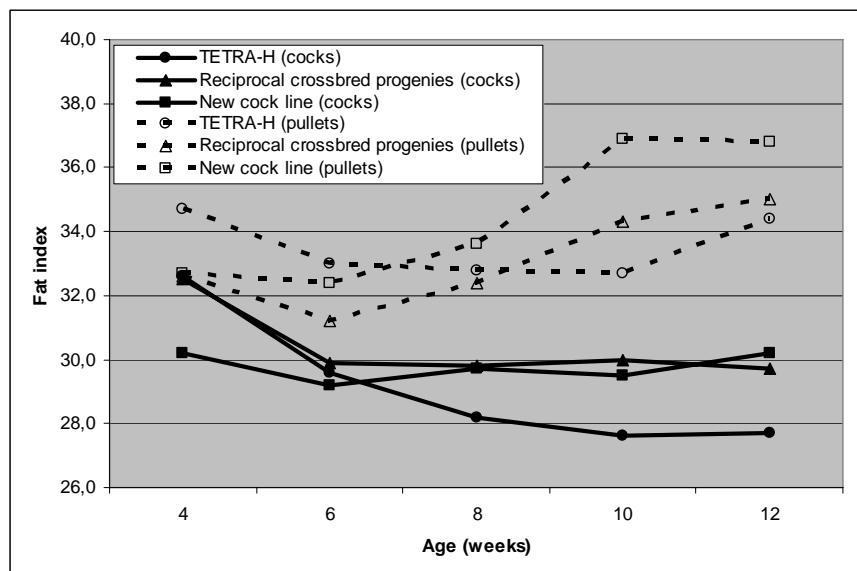
In the TETRA-H cocks the fat index was decreasing also from that time forth, while it remained at the same level in the other two genotypes. The lowest ratio of fat in the body was observed in the TETRA-H cocks at 12 weeks of age, which was lower by 6.7% and 8.3% than that of the other two genotypes. The difference between the TETRA-H cocks and cocks of the other two genotypes was also statistically significant at  $P<0.05$  level.

In the case of the pullets the fat index was decreasing till 10 weeks of age in the TETRA-H genotype, while it was increasing thereafter in the last two weeks of the experiment. In spite of this, the ratio of fat to the liveweight was increasing from 6

weeks of age in the other two genotypes and it reached its maximum level at 10 weeks age in the new cock line and at 12 weeks of age in the reciprocal crossbred progenies. The lowest value was reached also in this sex by the TETRA-H birds, which was lower by 1.7% and 6.5% than that of the other two genotypes. However, the differences between the examined genotypes were not statistically proven ( $P>0.05$ ) in this case.

**Figure 3**

**Changes in the fat index of TETRA-H chicks, reciprocal crossbred progenies and chicks from the new cock line between 4 and 12 weeks of age**



## CONCLUSIONS

Based on the results it was concluded that the new cock line seems to be good for increasing the liveweight of the TETRA-H chicks. However, when using these birds as parental line in the new breeding program, than it should be taken into consideration, that the higher body fat content of these animals could cause some unfavourable changes in the body composition of the newly developed birds. Therefore, the examination of changes in the body composition of the new animals seems to be necessary before using these birds widely in the breeding program.

## ACKNOWLEDGEMENT

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## Comparative study of the meat quality of common carp strains harvested from different fish ponds

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### ABSTRACT

*This study analyzed, whether there exist detectable differences among carp meat quality traits of fish harvested from different fish ponds at the age of 3 years. The slaughter and meat quality traits of altogether 40 carps was determined. It was stated that sex has a detectable effect on pH 24 and that body weight significantly affects flesh dry matter content. It was experienced that the water holding capacity of carp flesh is relatively low, and is expressedly sensitive towards induced effects like freezing and cooking.*

(Keywords: carp, meat quality)

### INTRODUCTION

Common carp is the dominant species in Hungary's fish production, with its share over 75%. This is largely mirrored in the national fish consumption habits as well, carp is the most popular fish in Hungary. In parallel with the alterations of the national consumption the proportion of processed products is increasing, giving a more expressed basis for the research concerning carp meat quality. Benthic zone is essential part of the fish pond biotop. Nutrients, organic material and micro-organisms are present here in a largely higher density than in the pond-water (Anvimelech *et al.*, 2002). The species in study, the common carp (*Cyprinus carpio L.*) is covering its nutrient demands mostly from the pond bottom thus it was hypothesized that its characteristics have a profound effect (directly via its consumption and indirectly through the water) on the carp meat quality.

Taking into account that carp consumption in Europe and North America is minimal or nil, relevant research results are rarely published in the leading scientific journals. Hancz *et al.* (1995) analyzed the alterations of carp body composition after natural and concentrated fish diet feeding regimes under laboratory conditions. Moreover Oberle *et al.* (1997) investigated the effects of divergent feeding protocols on the fillet composition, but fillet flesh quality was not involved. In the studies of Körnendi *et al.*, 2002; Romvári *et al.*, 2002; Lengyel *et al.*, 2001; Trenovszki *et al.*, 2008; Bauer *et al.*, 2009 mostly the body fat content, the fatty acid profile and the total body composition was determined. The production and meat quality of carp populations reared under different natural conditions has not yet been compared, thus, the present study aimed to perform such a comparison.

### MATERIALS AND METHODS

#### Fishes

Altogether 40 market-size common carp, *Cyprinus carpio*, were taken from four Hungarian fish farms. The carps were collected at the harvesting time (November-

December). Semi-intensive fish production in polyculture is performed at the farms, where the main species is the common carp. The main difference between farms is the geographical characteristics. First and second fish farms (TG1, TG2) are dam-like, built on clayey soil. The third and fourth (TG3, TG4) farms are in plains, built around banks. TG3 is on peaty soil, there is a large reedy marsh around the ponds; TG4 is on salty, alkaline soil. TG1 and TG2 are filled by streams, TG3 is filled with groundwater and TG4 is filled from the Tisza-river and by groundwater. Fish feeding is similar in each fish farm: 80% maize and 20% other grains.

Different carp strains were reared at farms: TG1: Attala mirror, TG2: Attala scaled, TG3: Hortobágy lean (scaled), TG4: Szeged mirror.

### **Slaughtering, slaughter value**

Carps were processed after percussive stunning in accordance with the rules of the *Carp Performance Testing Codex* (2001). Before the conventional processing, the following biometric traits were registered: body length, standard length, head length, tail length, body height, body width. After the processing the separated parts of the body were measured, and the slaughter value and fillet yield were calculated.

The following indexes were calculated: profile index (body length / body height), cross-section index (body height / body width), head index (body length / head length), tail index (body width / tail length)

### **Fillet flesh quality investigation**

Fillet pH was measured at 45 min and 24 h post mortem, by a Testo 205 precision pH meter (Testo AG, Lenzkirch, Germany). The colour (CIE Lab, L\*-lightness, a\*-redness, b\*-yellowness) of the fresh fillet was determined by a Minolta ChromaMeter 300 apparatus (Minolta, Osaka, Japan). Dripping loss was determined by the method of Honikel, (1998). To determine the so-called cooking loss, fillet samples (100 g) were closed into sealed bags and were cooked at 75 °C for 20 min. The exudate weight, as expressed in the percentage of the initial sample weight was referred to as cooking loss. The thawing loss was determined by the same manner, i.e. samples (25 g) were frozen (-20 °C) and thawed to room temperature after 2 days. Moreover, fillet dry matter content was determined by drying to constant weight at 103 °C.

### **NIRS analysis**

Carp fillet samples of ca. 25 g were freeze dried and ground on an IKA laboratory mill. Samples were filled into a small ring cup sample holder, and were scanned with a FOSS NIRSystem 6500 spectrometer (Foss NIRSystems INC., Silver Spring, MD) equipped with a sample transport module. Spectra were collected in reflectance mode between 800 and 2500 nm wavelength at 2 nm intervals, and were stored as log(1/R). Spectrum acquisition was performed with the WinISI II version 1.5 spectral analytical programme (InfraSoft International, Port Matilda, PA). All samples were scanned twice and the average spectra were used in the statistical analysis.

### **Statistical analysis**

SPSS 10 for Windows (1999) were used to the statistical analysis. First step was excluding outliers beyond double range of standard deviation. ANOVA with Tukey-test ( $P<0.05$ ) were used to compare the slaughtering and flesh quality parameters. Fixed factors were sex, strain and origin. The statistical analysis of NIR data was discriminant analysis, to classify samples according their origin, i.e. fish pond.

## RESULTS AND DISCUSSION

Table 1 shows the mean values and standard deviations in weight, slaughter weight, fillet yield, slaughter value, dry matter, profile index, cross-section index, head index, tail index, pH 45 min, pH 24 h, and cooking loss, dripping loss, thawing loss and colour parameters according to fish farms.

**Table 1****Slaughter and flesh quality parameters per fish farms/carp strains (mean±s.d.)**

Parameters	Attala mirror	Attala scaled	Hortobágy lean (scaled)	Szeged mirror
Weight	1499.6 ±141.5 <sup>b*</sup>	954.5 ± 303.9 <sup>a</sup>	1941.8 ± 269.5 <sup>c</sup>	1488.6 ± 209.3 <sup>b</sup>
Slaughtered weight	915.8 ± 90.6 <sup>bc</sup>	513.8 ± 188.1 <sup>a</sup>	1088.4 ± 137 <sup>c</sup>	844.4 ± 120.5 <sup>b</sup>
Fillet yield	45.7 ± 1.8 <sup>bc</sup>	40.1 ± 6.6 <sup>ab</sup>	43.7 ± 2.9 <sup>c</sup>	42.7 ± 1 <sup>b</sup>
Slaughtering value	61.1 ± 2.5 <sup>b</sup>	53.6 ± 6.6 <sup>ab</sup>	56.2 ± 2.8 <sup>a</sup>	56.7 ± 1.6 <sup>a</sup>
Profile index	2.1 ± 0.1 <sup>a</sup>	2.5 ± 0.2 <sup>b</sup>	2.5 ± 0.2 <sup>b</sup>	2.1 ± 0.1 <sup>a</sup>
Cross-section index	2.5 ± 0.2 <sup>a</sup>	2.5 ± 0.4 <sup>a</sup>	2.4 ± 0.1 <sup>a</sup>	2.5 ± 0.1 <sup>a</sup>
Head index	3.2 ± 0.1 <sup>a</sup>	3.7 ± 0.2 <sup>bc</sup>	3.7 ± 0.2 <sup>c</sup>	3.5 ± 0.2 <sup>a</sup>
Tail index	2.5 ± 0.3 <sup>ab</sup>	2.4 ± 0.3 <sup>ab</sup>	2.2 ± 0.2 <sup>a</sup>	2.5 ± 0.3 <sup>b</sup>
pH 45 min	6.4 ± 0.1	not determined	6.4 ± 0.2	not determined
pH 24 h	6.2 ± 0.1 <sup>a</sup>	6.4 ± 0.2 <sup>b</sup>	6.2 ± 0.2 <sup>a</sup>	6.4 ± 0.2 <sup>ab</sup>
Cooking loss	21.4 ± 1.6 <sup>b</sup>	16.1 ± 2.6 <sup>a</sup>	14.7 ± 2.2 <sup>a</sup>	17 ± 2.4 <sup>a</sup>
Dripping loss	2.4 ± 0.8 <sup>a</sup>	2.9 ± 0.6 <sup>a</sup>	2.1 ± 0.6 <sup>a</sup>	2.9 ± 1.2 <sup>a</sup>
Thawing loss	6 ± 1.6 <sup>a</sup>	5.1 ± 1.9 <sup>a</sup>	6.5 ± 1.8 <sup>a</sup>	6 ± 1.8 <sup>a</sup>
Dry matter	27.9 ± 3.4 <sup>a</sup>	27.5 ± 6.5 <sup>a</sup>	32.7 ± 4.9 <sup>a</sup>	29.1 ± 3.9 <sup>a</sup>
L	46.7±3.5 <sup>a</sup>	43.9±3.1 <sup>a</sup>	44.9±3.6 <sup>a</sup>	46.6±3 <sup>a</sup>
a*	2.6±1.2 <sup>a</sup>	3.2±1.8 <sup>a</sup>	3.8±1.4 <sup>a</sup>	2.9±1.4 <sup>a</sup>
b*	1.5±1.2 <sup>b</sup>	0.8±1.4 <sup>ab</sup>	0.9±1.1 <sup>ab</sup>	0.1±0.9 <sup>a</sup>

<sup>a,b</sup> P<0.05

According to our possibilities fish groups from the different farms were selected to provide maximum homogeneity, but due to the largely different local conditions in the technology and natural circumstances the slaughter weight was considerably different among groups. Sex had a defined effect on the weight, namely females largely exceeded males, most probably due to the well developed ovaries. By analysing slaughter value the prescriptions of the *Carp Performance Testing Codex* (2001) were followed and the slaughter value showed large differences among farms. This trait of carps originating from farm TG1 showed the highest values. Interestingly fillet samples in this group showed the highest cooking loss, referring to a low water holding capacity of carp flesh.

The somatometric indices of fish are basically connected to the strains. However, mirror and scaled strains showed a clear significant difference only in the profile index. It is an intriguing result that the water holding capacity of carp flesh is relatively low (independently of farm), total losses (cooking, thawing and dripping) ranging from 23 to 30%, leading to the conclusion that the culinary processing is best when using only mild treatment conditions, mostly on fresh fillet. When comparing our results to those of other freshwater species, it seems to be relatively high; in case of African catfish Szabó *et al.* (2009) described ca. 12% for the total losses. In the catfish, similarly to the carp the induced losses (cooking and thawing) were rather high, as compared to the spontaneous dripping.

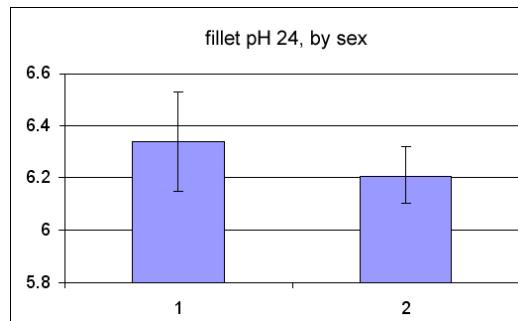
The colour of meat is basically determined by its hem pigment contents, in particular by myoglobin and haemoglobin. Carp flesh is low in myoglobin and is classified as white meat. Results in our study concerning colour (L, a\*, b\*) are characteristic to carp flesh. The fillet of female individuals was in every case characterized by higher L values, as compared to the males ( $46.7 \pm 2.75$  vs.  $45.1 \pm 3.54$ ,  $P < 0.05$ ). According to *Gregory and Grandlin* (1998), in poultry paler meat colour is associated with lower ultimate pH, which is consonant with our results.

The pH value measured at 45 minutes *post mortem* was always only slightly higher than at 24 h p.m. This was caused by the muscle to meat conversion, by the breakdown of glycogene. The relatively mild pH fall is attributed to the low glycogene concentration of fish flesh, as compared to mammals. Interestingly, a defined sex effect was found on the fillet pH, namely in the entire dataset the male individuals had significantly higher fillet pH values, as compared to the females (*Figure 1*).

The NIRS analytical results indicate that simple classification of ground, freeze-dried samples is effective, when performing the analysis on the entire spectral interval (*Table 2*).

**Figure 1**

**Sex-dependent difference in the pH 24 value of carp fillets (1-male, 2-female)**



**Table 2**

**Classification results of the 40 carp fillet samples, based on NIR spectra, by discriminant analysis**

Attala mirror	18	0	0	1
Nagyberki scaled	0	20	0	0
Hortobágy lean	0	0	17	1
Szeged mirror	0	0	1	15
Total	18	20	18	17
Misses	0	0	1	2
Succesfully classified	18	20	17	15
Standard error of cal.	0.21			
R <sup>2</sup>	0.75			
Standard error of cross val.	0.24			
1-VR	0.69			

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## Subjective quality of yogurts of two Slovene private and two national brands having equal objective quality

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### **ABSTRACT**

*In order to compare the subjective quality of yogurts sold on the Slovene market under private and national brands, 100 randomly selected consumers were asked to taste samples of yogurts presumably belonging to two Slovene private brands (PB1 and PB2) and two Slovene national brands (NB1 and NB2). The samples offered to participants did not differ in terms of objective quality (the same product was placed in all 4 samples). The participants assessed their quality by assigning to each sample a mark between 1 and 7 (1 meaning the lowest quality, 7 the highest). The average value of marks assigned to samples of presumably national brands was higher (5.61 for NB1 and 5.26 for NB2) than the average value of marks given to samples of presumably private brands (4.85 for PB1 and 4.72 for PB2). The paper discusses the possible reasons for such perception of brands and the potential consequences of the findings for milk producers.*

(Keyword: brand, subjective equality, objective quality, yogurt)

### **INTRODUCTION**

Considering the ex-farm gates value, milk production is the most important agricultural activity in Slovenia. The annual milk production quota set for Slovenia is currently over 550 million liters. With a buying-in price of EUR 0.30/l, such amount totals EUR 165 million. The buying-in price of milk that dairies pay to producers depends, among others, on how much the consumers are willing to pay for dairy products.

In Slovenia, as well as on other European and world dairy markets, consumers are able to choose between products of national brands and those produced under private brands. The market share of private brand products is increasing (*Private label today*, 2007). Some data indicate (*Pohar and Klopčič, 2010*) that for producers producing both groups of products, the production of private brand products is less profitable than the production of products under their own brands. The reason for such is that the purchase point price for private brands is not formed by marking-up the processor's price, but by subtracting the retailer's costs from the purchase point price which is set by the retailers themselves. Considering the above, the increasing market share of private brands in the dairy sector would mean lower profitability for certain processors, which can only be preserved if the prices at which milk is purchased from the producers are reduced. Therefore, a decrease in the market share of national brand products is not in the interest of milk producers. The complex issue of price transparency throughout the entire supply chain has been pointed out also by the European Commission (*European Commissions, 2008*).

The consumers' decision to buy a certain product also depends on the position such product occupies in their minds (*Ries and Trout, 1986*). One of the factors defining such

position is indeed the product's quality. Yet quality is a rather complex term, which is particularly true of food products (*Acebron and Dopico*, 2000). *Grunert* (1995) for example distinguishes among product-oriented quality, process-oriented quality, and users-oriented quality. He combines the first two into what is known as »objective« quality, as it may be measured on the product as such, whereas users-oriented quality may only be measured on the users and is therefore referred to as »subjective«. The author stresses, however, that all three types of quality are closely interrelated.

For certain types of products, differentiation based on objective quality is not easy and additional indicators known as extrinsic quality cues (price, brand name, place of origin, promotion, packaging, presentation) are needed, which are determined by marketing efforts (*Steenkamp*, 1990). *Olson* (1977) describes extrinsic quality cues as characteristics that are related to the product but are not a physical component thereof. In the context of the marketing mix concept known as 4P (*Pride and Ferrell*, 1997), which consists of four elements namely product, price, promotion, and place, *Kotler* (1988) distinguishes between the core of the product and other layers (design, primary and secondary packaging, guarantee, etc.). Thus, objective quality could be defined as the part of the product that *Kotler* (1988) considers to be the core of the product.

Our research focused on the subjective quality assigned by consumers to yogurts of different brands that do not differ in terms of objective quality and are also considered equal as regards other characteristics of extrinsic quality cues.

## MATERIALS AND METHODS

The research involved liquid yogurt with 3.2% milk fat, with no aromas, fruit pulps or probiotic cultures added. This product was selected for the following reasons: the product is generally well known, quantitatively important within the category of fermented dairy products, and is not a niche product. It can be found on the shelves of two national retail chains under their own brands, along with a similar product sold under the brand names of two Slovene dairies. Both retail chains as well as both dairies can be considered market leaders. The actual technology of production of such products does not allow for great differentiation among the products at the core level. Both dairies produce yogurts under their own brands and as private brand products. It can be therefore speculated that private brand products and national brand products purchased by consumers on a daily basis do not largely differ in terms of objective quality.

The research was conducted among the students of the Ljubljana University. 100 randomly selected students were asked to assess the quality of the samples offered and evaluate them with a mark between 1 and 7, 1 meaning the lowest quality and 7 the highest. The samples were provided in equal 100 ml cups. Two cups were designated as private brands (Private Brand 1, Private Brand 2), while the other two were designated as national brands (National Brand 1, National Brand 2). The designations were not provided in the original typography and color but were similarly neutral for all four samples. Thus, the evaluation could not be affected by design – one of the extrinsic quality cues. Moreover, the objective quality of all samples was exactly the same since each of the participants' four cups was filled with the same yogurt. This means that the research participants actually tasted a yogurt of the same objective quality although they believed they were evaluating yogurts of two national and two private brands. The differences in the marks assigned to the samples thus reflect the evaluation of the extrinsic quality cue element known as the brand.

After evaluating the samples, the participants were asked to tell us which yogurt would they buy if there was no difference in the price, and to explain their expected buying decision.

## RESULTS AND DISCUSSION

*Table 1* presents the average values and standard deviations of marks assigned by research participants to each of the four samples, as well as their buying preferences for each sample.

**Table 1**

**Mean value and standard deviation of scores given to brands and number of times brand was selected as preferred buy**

<b>Brand</b>	<b>Average score</b>	<b>Standard deviation</b>	<b>Number of times selected as preferred buy</b>
National brand 1 (NB1)	5.61	1.26	59
National brand 2 (NB2)	5.26	1.31	28
Private brand 1 (PB1)	4.85	1.17	4
Private brand 2(PB2)	4.72	1.28	9
NB1+ NB2	5.50	1.29	87
PB1+ PB2	4.76	1.22	13

Results show that higher values were assigned to yogurts presumably produced under national brands. In *Table 2* probabilities that two average values under consideration do not differ are presented.

**Table 2**

**Probabilities of hypothesis  $\mu_i = \mu_j$**

	<b>NB2</b>	<b>PB1</b>	<b>PB2</b>	<b>PB1+ PB2</b>
NB1	0.2148	<0.001	<0.001	
NB2		0.0881	0.0167	
PB1			0.8746	
NB1+NB2				<0.001

From this table it can be seen that the difference between marks obtained by the samples Private Brand 1 and Private Brand 2 was not statistically significant. Likewise, there was no statistically significant difference between National Brand 1 and National Brand 2, while there was statistically significant difference between National Brand 1 and Private brand 1 as well as between National Brand 1 and Private brand 2. There was also statistically significant difference between National Brand 2 and Private brand 2. Considering the values for national brand and private brand samples in total, it may be concluded that research participants assigned statistically higher marks to national brand products. Thus, national brand products on average obtained better results although all tasted yogurts were of absolutely equal objective quality.

According to *Grunert* (2002), the brand is a particular type of extrinsic quality cues. Nowadays, in evaluating the quality of a product offered under a renowned brand, what also matters is consumers' past experience with such brand, not just the objective quality of the product at the time of consumption. Obviously, also in our case the evaluation of samples was affected by experience the research participants had with the

selected brands. If this had been irrelevant, the samples would not have been given different marks. Yet experience is not necessarily and exclusively related to objective quality. The participants tasted a product for which – to our opinion – the differences between brands in terms of objective quality, if any, are probably the smallest compared to other dairy products (with the exception of UHT and fresh milk). The products sold under private brands for a specific retail chain are in fact produced by the same companies that on the market feature also with their own brand products.

The evaluation could thus depend on experience with other products offered on the market under the same umbrella brand. According to *Podnar et al.* (2007), the image of a certain product placed on the market under an umbrella brand is defined also by other products sold under the same umbrella brand. If the latter corresponds to the name of the corporation, the image of such product also reflects the image of the corporation. In our case, the four products offered for evaluation feature on the market under umbrella brands that are identical to the names of the corporations.

Looking at the results from such point of view and considering the potential buying preference, there seems to be a significant difference even between the two national brands; the product under National Brand 1 would be preferred in 59 cases, while the product under National Brand 2 would be chosen in 28 cases. Altogether, only 13% of research participants would prefer private brands.

Also interesting is what they stated as reason why they preferred a certain product. The results are indicated in *Table 3*.

**Table 3**

**Reasons stated by participants why a specific product was chosen**

<b>Reasons for selection</b>	<b>Number of times product was selected for this reason</b>
I like the taste	47
I know the brand	21
Has the right acidity	15
I known product	5
Product comes from town I live in	4
I like the thickness	3
I like package of product	2
It has the highest quality	1
I usually buy in the shops of this chain	1
I selected it because of trend	1

Over 50% of participants said the reason for their decision was something related to the objective quality of the sample tasted. This category comprised replies such as »taste«, »acidity«, »thickness« and »quality«. Yet it should not be overlooked that a large share of participants also said the reason to prefer a certain product to be knowing of »brand«, »product«, and »origin«, i.e. attributes which do not directly relate to »objective« quality. One also needs to consider that the participants expressed their buying preferences on the assumption that the price of all products was the same. Private brand products are usually cheaper than national brands, which mean that in their everyday life the participants might not opt for the product they ranked higher or for which they expressed buying preference.

The question is to what extent the difference in the evaluation of specific products can be attributed to the reasons stated above, i.e. the participants' experience with the „real” similar products of the selected national and private brands, and experience with other products of the same umbrella brands. Considering the discussions about price transparency in the food chain held in 2009 also in Slovenia, it could be assumed that the difference in evaluation might even be related to consumers' values. Brunsø *et al.* (2005) argue that the consumers' perception of quality of a certain product may be established as a relation between the product's qualities and certain rather abstract, more central cognitive categories, such as values. Here, the product's qualities are no longer relevant as such but only in relation to the fact that they cause certain desired or undesired consequences. The relevance of such consequences is therefore defined by consumers' values. A recent opinion in Slovenia (Kuhar, 2009) is that as regards food products, the share of value added pertaining to retailers is disproportionately large compared to the share of value added that pertains to processors and producers, which makes the distribution of value added unjust.

## CONCLUSIONS

If other segments of consumer would evaluate the quality of national and private brands similar, as established in our research, it could mean that the dairy industry in future would not increase its demands for lower buying-in milk prices. But such situation could change rather fast.

On long term Slovene dairy industry can only survive if production costs are equal to those of the European competition. The same is true for milk producers. The subjective quality of Slovene private brand products will not change if such products are no longer produced by Slovene dairies. Domestic retailers will search for non-local suppliers of products to be sold under their private labels in the case the prices of dairy products produced by local dairies would be too high. Likewise, the subjective quality of products of national dairy brands will remain the same even if products are not produced from milk of Slovene producers. This means that Slovene dairies will not produce their national brands from milk of local milk producers.

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## The influence of disposal technology obtained with alkaline treatments on D-amino acid content of slaughterhouse waste

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### **ABSTRACT**

*In our experiment the change in D-amino acid content of slaughterhouse waste due to the treatments was examined. The treatments were done with sodium and potassium hydroxide solution, respectively, for 2, 3 and 6 hours at 135, 150 and 153 °C. Summarized, it can be said that due to the heat and alkali combinations we used aspartic acid, glutamic acid, tryptophan and isoleucine transform in 40–50% into the D-isomer. Even though the hydrolysed product obtained this way met in other parameters the requirements of the modern feeding, one should be expect that most of the amino acids undergo full racemization during this process.*

(Keywords: slaughterhouse waste, waste treatment, alkaline hydrolysis, racemization of amino acids, D-amino acids)

### **INTRODUCTION**

With the appearance of BSE Europe faced a huge problem, as processing of waste material that can be brought into connection with BSE is strictly forbidden. By the introduction of the new EU regulations (EU Directive 1774/2002) slaughterhouse waste has been categorized into three categories (high, medium and low risk) and conditions of waste treatment were defined. As a result of the restrictions the protein demand of animal breeding considerably increased and also the demand for elaborating new methods for the treatment of waste of animal origin. The alkaline hydrolysis of animal proteins has been applied in the United States at the Albany Veterinary School since 1993 (Kaye *et al.*, 1998). There are several results showing that alkaline hydrolysis deconstructs the prions (Tagochi *et al.*, 1991; Ernst, 1993; Taylor *et al.*, 1999; Taylor, 2000), therefore it proved to be especially effective in the treatment of materials of animal origin of the highest risk. The alkaline treatment is a procedure during which due to the alkaline medium the high molecular proteins disintegrate into smaller peptides, free amino acids. This is a very important step e.g. in the disintegration of prion proteins causing BSE.

It was obtained in earlier investigations that every impact involving high temperature alkaline treatment results in racemization of most of the amino acids (Man and Bada, 1987; Friedman, 1991; Imai *et al.*, 1996). When treating the proteins with alkali at low temperature or the hydrolysis is carried out at high temperature in neutral or acidic medium, this can also cause racemization, but the combination of alkaline treatment and high temperature results in racemization almost surely.

In the course of our research the racemization of amino acids including epimerization of isoleucine was examined in the products of an experiment targeting to render harmless slaughterhouse waste.

## MATERIALS AND METHODS

### Treatment of the samples

In the experiments ox brain samples obtained from Croatia were used. The samples were stored at -20 °C, then after defrosting they were homogenized and divided into 400 g parts. During treatments to 400 g of sample 600 cm<sup>3</sup> of distilled water and 44 cm<sup>3</sup> of 45% KOH or 30 cm<sup>3</sup> of 45% NaOH solution was added. Accordingly, to the control sample 644 and 630 cm<sup>3</sup> of distilled water was added. The alkaline mixtures were heated at different temperatures and under different pressures (135 °C, 2.75 bar; 150 °C, 4.78 bar and 153 °C, 5.17 bar) and after 2, 3 and 6 hrs sample was taken from the reactor. Out of each treatments the measurements were carried out in 3 repetitions. Along with the control altogether 19 treatments were carried out. (The samples: N1, N2, N3, N4, N5, N6, N7, N8, N9 and K1, K2, K3, K4, K5, K6, K7, K8, K9; N = hydrolysis with NaOH, K = hydrolysis with KOH).

### Protein hydrolysis conditions for amino acid analysis

In order to release the amino acids the hydrolysis was carried out using 6 M hydrochloric acid. As tryptophan decomposes under acidic conditions and alkaline conditions lead to racemization, we applied a 3 M p-toluenesulfonic acid solution containing 3-indolyl-propionic acid during the determination of the Trp. The protein/3-indolylpropionic acid ratio was set to the value of 1:1 (*Liu and Chang*, 1971; *Gruen and Nichols*, 1972). In both cases the hydrolyses were carried out in closed ampoules under nitrogen atmosphere at 110 °C for 24 hrs. Th pH of the hydrolysates was set with 4 M NaOH solution.

### Derivatization and analysis of amino acid enantiomers

From the amino acid enantiomers during precolumn derivatization with OPA (o-phthaldialdehyde) and TATG (1-thio-β-D-glucose-tetraacetate) diastereoisomers were formed (*Einarsson et al.*, 1987; *Csapó et al.*, 1995). The derivatization and analysis were carried out using a MERCK-Hitachi HPLC apparatus. Diastereomer derivatives of Asp and Glu were separated on a 125 mm×4 mm i.d. Superspher 60 RP-8e column. For the analysis of the Trp enantiomers a 125 mm×4 mm i.d. Purospher RP-18e column was used. The derivatives were detected by fluorescence detector (ex.: 325 nm, em.: 420 nm). L-isoleucine and D-allo-isoleucine content was determined using an INGOS AAA400 amino acid analyzer. Separation took place on a 350×3.7 mm, OSTION Lg ANB cation-exchange column.

## RESULTS AND DISCUSSION

During our research ox brain samples obtained from Croatia and treated with NaOH and KOH, respectively, at different temperatures for different durations, were analyzed. As a result of the treatments the concentration of the individual amino acids in the samples increased compared to the starting materials. The reason for this was that during the treatments the samples lost some amount of solvent and by this they become more concentrated.

Aspartic acid, glutamic acid, isoleucine and tryptophan enantiomers of the hydrolysate were determined. Aspartic acid and glutamic acid were chosen because proteins used in animal feeding contain relatively much of these two amino acids, in some cases their total amount can reach even 30–40% of the crude protein content. Isoleucine was chosen because the determination of D-allo-isoleucine formed due to the alkaline treatment does not require

special analytical procedure, it can be easily analyzed by an automatic amino acid analyzer. Tryptophan was chosen because it is an essential amino acid on the one hand, and the alkaline hydrolysis conditions are favourable for avoiding the decomposition of tryptophan.

### Racemization of aspartic acid and glutamic acid

It was established that even during the hydrolysis carried out at the lowest temperature and for the shortest time (135 °C, 2 hrs) more than 40% of both amino acids racemized. Proportion of the D-amino acids is expressed by the formula of  $D/(D+L) \times 100$  (*Table 1.* and *Table 2*). After analysis of the control, not heat-treated sample for D-aspartic acid 3.9% and for D-glutamic acid 1.6% was obtained which could be attributed to the intervention prior to the heat treatment and to the racemization occurred during sample preparation and protein hydrolysis, respectively.

It was observed that at the two higher temperatures, during longer treatment the concentration of the amino acids decreased despite the solutions becoming more concentrated the concentration of the amino acids decreased, reason for which was presumably the decomposition of Asp and Glu.

**Table 1**

#### Extent of racemization of aspartic acid due to alkaline treatments

Marking	N1	N2	N3	N4	N5	N6	N7	N8	N9
$\frac{D}{D+L} \times 100$	43.0%	43.4%	43.9%	44.0%	44.6%	45.5%	44.2%	44.3%	44.8%
Marking	K1	K2	K3	K4	K5	K6	K7	K8	K9
$\frac{D}{D+L} \times 100$	43.2%	43.4%	43.7%	44.1%	44.3%	44.7%	44.3%	44.5%	44.9%

**Table 2**

#### Extent of racemization of glutamic acid due to alkaline treatments

Marking	N1	N2	N3	N4	N5	N6	N7	N8	N9
$\frac{D}{D+L} \times 100$	43.6%	44.0%	44.7%	45.4%	46.6%	45.1%	45.9%	45.9%	46.1%
Marking	K1	K2	K3	K4	K5	K6	K7	K8	K9
$\frac{D}{D+L} \times 100$	45.5%	45.4%	45.4%	44.2%	45.6%	45.6%	45.7%	45.8%	46.0%

### Racemization of tryptophan

In case of tryptophan it was necessary to employ another hydrolysis method because tryptophan completely decomposes during the 6 M hydrochloric acidic hydrolysis for 24 hrs due to cleavage of the indole group. During the acidic hydrolysis we applied, using the protecting agent more than 80% of tryptophan could be recovered from the protein. The extent of racemization for Trp is given in *Table 3*. It was found that 39–45% of tryptophan racemized during the heat treatment. Like in case of aspartic acid and glutamic acid, treatment at higher temperature and longer treatment time, respectively, led to decomposition of tryptophan.

**Table 3****Extent of racemization of tryptophan due to alkaline treatments**

<b>Marking</b>	<b>N1</b>	<b>N2</b>	<b>N3</b>	<b>N4</b>	<b>N5</b>	<b>N6</b>	<b>N7</b>	<b>N8</b>	<b>N9</b>
$\frac{D}{D+L} \times 100$	40.0%	40.6%	41.5%	36.8%	40.4%	40.4%	43.8%	44.6%	44.3%
<b>Marking</b>	<b>K1</b>	<b>K2</b>	<b>K3</b>	<b>K4</b>	<b>K5</b>	<b>K6</b>	<b>K7</b>	<b>K8</b>	<b>K9</b>
$\frac{D}{D+L} \times 100$	43.5%	43.7%	46.2%	40.2%	40.5%	40.2%	36.8%	41.4%	41.2%

**Epimerization of isoleucine**

It was found that the control sample did not contain D-allo-isoleucine even in traces. The extent of the racemization is shown in *Table 4*. Based on the results it can be said that in the treated samples the total amount of the isomers practically does not change. In contrast with the other three examined amino acids, in case of isoleucine we do not have to reckon with the decomposition of the amino acid.

Examining the extent of the racemization it was established that carrying out the treatment at 135 °C with NaOH, the extent of the epimerization of Ile is less than in the other treatments, although it is above 40% also in this case. In the other treatments the racemization can be considered as complete.

**Table 4****Extent of epimerization of isoleucine due to alkaline treatments**

<b>Marking</b>	<b>N1</b>	<b>N2</b>	<b>N3</b>	<b>N4</b>	<b>N5</b>	<b>N6</b>	<b>N7</b>	<b>N8</b>	<b>N9</b>
$\frac{D}{D+L} \times 100$	42.2%	42.4%	43.5%	49.8%	49.4%	49.8%	48.7%	48.3%	48.7%
<b>Marking</b>	<b>K1</b>	<b>K2</b>	<b>K3</b>	<b>K4</b>	<b>K5</b>	<b>K6</b>	<b>K7</b>	<b>K8</b>	<b>K9</b>
$\frac{D}{D+L} \times 100$	48.9%	48.9%	49.6%	49.8%	49.6%	49.6%	49.4%	49.4%	48.6%

**CONCLUSIONS**

Evaluating the results for the four amino acids it was established that during the alkaline treatment each amino acid racemized in 42–46%, hence in practical respect the obtained material can be considered as racemic mixture of the amino acids. The amino acid content of the samples decreased only in the treatment carried out at the highest temperature for the longest time. As the racemization can be considered as complete even in the treatment carried out at the lowest temperature and for the shortest time and since racemization is a result of the roughest technological interventions, we suppose that a treatment at 135 °C for 2 hrs with NaOH or KOH is sufficient for the entire destruction of the protein structure.

Based on the above the almost complete racemization makes the obtained material unfit for being used as animal feedstuff since the higher animals – with the exceptions of ruminants – can utilize only L-amino acids, D-amino acids act as growth inhibitor. Nothing seems to be against, however, that the product of the hydrolysis with KOH, after neutralization, is used as nitrogen fertilizer in the soil.

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## Evaluation of biological value of wheat sprout: fat content, fatty acid composition

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### ABSTRACT

During our research work fatty acid content of the wheat and wheat sprout was investigated during the germination. It was found that both the saturated and unsaturated fatty acids hardly changed during the germination. The most important saturated fatty acid of the sprout investigated by us is palmitic acid, amount of which hardly changed or even increased during germination. Oleic acid and linoleic acid were present in the highest concentration among the unsaturated fatty acids in the sprout investigated. The concentration of oleic acid remained unchanged during the germination period, and the same applies to linoleic acid. Based on our investigation it can be stated that most of the fatty acids hardly changed during the germination, and there was no verifiable tendency.

(Keywords: sprout, chemical changes during germination, fat content, fatty acid composition)

### INTRODUCTION

Sprouting is a natural biological process that every higher plants exhibit, during which the seed at rest starts to grow under favourable environmental conditions (appropriate moisture content, temperature, oxygen) and a new plant develops. Germination can lead to the development of such functional foods that have a positive effect on the human organism and that help in maintaining the health (Sangronis and Machado, 2007).

The sprouts fulfill the requirements of the modern nutritional science for whole-food. Researches found that the sprouts are a good source of ascorbic acid, riboflavin, choline, thiamin, tocopherol and pantothenic acid (Lintschinger *et al.*, 1997).

Urbano *et al.* (2005) examined the protein digestibility of various sprouts and bioavailability of the minerals, Gill *et al.* (2004) the relationship between the consumption of vegetables and the prevention of cancer, and Clarke *et al.* (2008) the efficiency of the sulforaphane content of different sprouts in cancer prevention. Kim *et al.* (2004) examined the change in fatty acid composition due to sprouting. It was established that majority of fatty acids of buckwheat is unsaturated ones, out of which linoleic acid can be found in the highest amount. Tokiko and Koji (2006) examining fat content and fatty acid composition of various sprouts established that the fat content ranged between 0.4 and 1.6%. In the course of fatty acid content analysis it was found that linolenic acid was present in the highest concentration, 23% in case of buckwheat, 48% in the soybean, 47.7% in the clover and 40.6% in the pea.

Studying the literature, we found no more data on the change in fat content and fatty acid composition during sprouting. Because of the above we started our

investigation relating to fatty acid composition of wheat sprout and the changes in the fatty acid composition due to germination. During our work we determined the fatty acid composition of wheat sprout and its change in the function of germination time.

## MATERIALS AND METHODS

### The examined samples, germination

Commercially obtainable organic wheat were obtained. The seeds were washed in 0.1% H<sub>2</sub>O<sub>2</sub> for 1 min then soaked in distilled water for 24 hrs. After the 24 hrs elapsed, the seeds were placed into germination bowls, and germinated at 20 °C in a Memmert 200 incubator. They were rinsed twice a day with distilled water and samples were taken in every 24 hrs. According to the domestic practice and international recommendations wheat was germinated for 3 days. After germination the sprouts were washed with distilled water, dried at 60 °C, then stored frozen at -10 °C until the analyses.

### Determination of fat content and fatty acid composition

Crude fat content of the sprouts were determined in a Soxhlet extractor after extraction with diethylether according to the Hungarian Standard (HS 6830/19-79). Determination of the fatty acid composition was performed using a Varian 3800 gas chromatograph. Results were given as fatty acid methyl ester relative weight%.

## RESULTS AND DISCUSSION

*Table 1* shows crude fat content of the starting seed and the sprout. Results were given in weight% on air dry matter basis.

**Table 1**

### Crude fat content of the wheat seed and wheat sprout

Number	Description	Crude fat content (%) (on air dry-matter basis)
1	Wheat seed	1.7
2	Wheat sprout, day 3	1.7

In case of wheat no change was experienced between seed and sprout. *Table 2* shows fatty acid composition of wheat seed and wheat sprout.

In the wheat sprout the fatty acids being present in the highest concentration are palmitic acid, linoleic acid and oleic acid. Out of the saturated fatty acids palmitic acid is present in 33.5% in the wheat sprout which value is higher than that for the wheat seed (31.2%). Accordingly due to the germination the concentration of palmitic acid increased. The value for stearic acid decreased from 1.9% in the seed to 1.2% in the wheat sprout. Out of the saturated fatty acids beyond the mentioned ones also undecanoic acid (1.7% in the wheat seed and also in the sprout), lauric acid (0.1% in both of the samples), tridecanoic acid (0.7% in the seed and the sprout), myristic acid (0.8% in the wheat seed and 0.5% in the wheat sprout), pentadecanoic acid (0.3% in both samples), arachidic acid (0.3% in the seed and 0.2% in the sprout) and behenic acid (1.2% in both samples) could be detected in the samples.

**Table 2****Fatty acid composition of wheat seed and wheat sprout**

Fatty acid	Wheat seed	Wheat sprout, Day 3
	Fatty acid methyl ester %	
Undecanoic acid 11:0	1.7	1.7
Lauric acid 12:0	0.1	0.1
Tridecanoic acid 13:0	0.7	0.7
Myristic acid 14:0	0.8	0.5
Pentadecanoic acid 15:0	0.3	0.3
Palmitic acid 16:0	31.2	33.5
Stearic acid 18:0	1.9	1.2
Oleic acid 18:1	10.7	7.8
Linoleic acid 18:2	25.6	27.3
Arachidic acid 20:0	0.3	0.2
Eicosenoic acid 20:1	0.9	0.4
$\alpha$ -Linolenic acid 18:3n3	2.0	2.5
Behenic acid 22:0	1.2	1.2
Eicosatrienoic acid 20:3n6	1.4	1.5
Eicosatrienoic acid 20:3n3	2.3	0.2

Out of the monounsaturated fatty acids oleic acid is present in the highest concentration, its value decreased from 10.7% in the wheat seed to 7.8% due to germination. In the samples also eicosenoic acid could be detected: 0.9% in the wheat seed and 0.4% in the wheat sprout.

Out of the polyunsaturated fatty acids linoleic acid was present in the highest concentration, with 25.6% in the starting wheat seed and increasing to 27.3% in the sprout. In the samples also  $\alpha$ -linolenic acid (2.0% in the wheat seed and 2.5% in the sprout) and eicosatrienoic acid (C20:3n6): (1.4% in the wheat seed and 1.5% in the wheat sprout), (20:3n3): (2.3% in the wheat seed, 0.2% in the sprout) were detectable.

By the analysis of the fatty acid composition of wheat sprout, we established that by far no such radical changes occurred during the germination as reported by *Kim et al.* (2004), and *Tokiko and Koji* (2006). Calculating on dry-matter basis the crude fat content of the sprouted plant either did not change or decreased during the sprouting.

Regarding the fatty acid composition, concentration of palmitic acid, the saturated fatty acid being present in the highest concentration, increased in case of wheat or did not change during the germination. Similar change can be reported in case of stearic acid, and we cannot give a definite answer to how the stearic acid content changed during the germination. It is very probable that either the stearic acid content or palmitic acid content does not suffer a substantial change due to the germination.

We can formulate almost similar tendencies in case of unsaturated fatty acids. In case of wheat sprout the amount of oleic acid decreased somewhat. The amount of linoleic acid remains practically unchanged in the germination period. The other polyunsaturated fatty acids occur in such a small concentration, that even the tendency of the changes is difficult to follow.

Summarized, it can be established that some of the saturated fatty acids decrease minimally, others remain unchange. Out of the unsaturated fatty acids oleic acid and

linoleic acid practically hardly changes. From our investigations we can conclude that in case of the wheat sprout the germination process hardly affected the fatty acid composition of the fat of the wheat plant, consequently the biological value of the fat. We cannot confirm the results found in the literature reporting that due to the germination the amount of the saturated fatty acids considerably decreases and the amount of the polyunsaturated essential fatty acids considerably increases.

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