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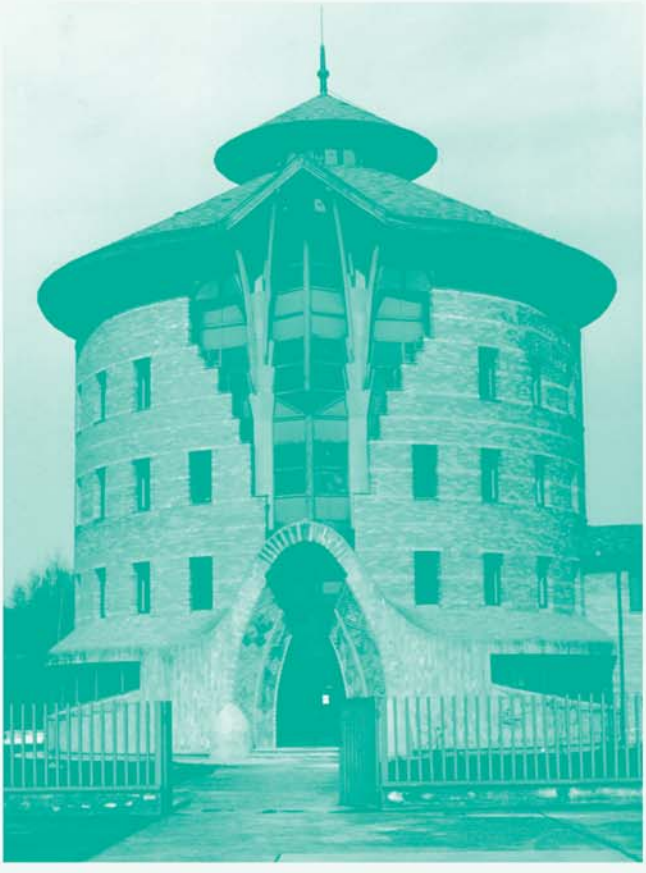

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Acta Agraria Kaposváriensis

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“Animal Science Days”

FUTURE TRENDS OF RESEARCH ON FOOD QUALITY AND SAFETY

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Establishing new food safety approach in EU accessing countries – Croatian challenges and opportunities

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ABSTRACT

One of the basic goals of the Croatian agricultural policy is to enable consumers the access to the adequate and stable food offer that complies with their requirements, especially regarding food safety. Croatian National Food Safety Strategy, based on new EU food safety approach, is currently in the phase of discussing drafted material. Official food safety framework is being redesigned in the aim to improve effectiveness by introducing the process of risk analyses. Coordination and cooperation between the competent authorities involved in food safety issue is becoming more intensive due to the start of negotiation process with EU. Personnel and institutions involved in food safety control are, through pre-accession programs, increasing their knowledge and abilities. Such harmonization of approaches and standards within all the EU member countries is required in order to guarantee same protection to all consumers. The paper discusses challenges and opportunities that Croatia is facing during the EU pre-accession period.
(Keywords: food safety, Croatia, EU, risk analysis, strategy)

INTRODUCTION

Every country needs an effective food safety program in order to protect the health of nation and to participate in international food trade. The goal of such program is to ensure safety and quality of the total food supply, leading to reduction in the incidence of food-borne diseases and improvements in nutritional status and quality of life. Under national food safety programs, national infrastructures and problems can be systematically reviewed and used as a base for development of strategic plan for food safety. The status and delivery mechanisms for food safety programs varies from country to country, reflecting the different stages of development, variations in food safety problems and various administrative arrangements.

Pan-European Conference on Food Safety and Quality (*FAO/WHO*, 2002) held in Budapest set up requests from countries in South East Europe to strengthen food control and legislation. The importance of providing a platform for information exchange and facilitating a regional approach was stressed on the Conference. EU integration process is gathering different cultures, eating habits and perceptions on food safety. It is obvious from the experience, after the last accession of ten new EU countries, that this will not be easy process and certain period will be needed to establish unique criteria for all the food standards in praxis. It is necessary that developed countries assist the EU accessing countries in establishing modern food safety control system in order to give them opportunity to compete on international market under the same conditions. The following steps are considered as the most important to achieve this goal:

- Drafting national food safety strategy;
- Transposition of EU legislation to national food safety legislation;
- Improvement of the institutional framework;
- Implementation and permanent audit of implemented measures;
- Permanent training in key elements of modern food control system.

Governments in the EU accessing countries are expected to strengthen sub-regional cooperation through enhancement of national food safety authorities making them capable to ensure necessary consumers' protection. Harmony within and between systems in different countries of European region, beside increasing of consumers' protection, should erase unnecessary trade barriers (*WTO*, 1995a, 1995b). The Republic of Croatia commenced trade integration processes by becoming WTO member in the end of the year 2000, and continued this process by concluding free trade agreements with almost all European countries. The Stabilization and Association Agreement enabled Croatia to use duty-free approach to the EU market for nearly all agro-food products. By becoming full EU member country, Croatia will step to the market with around 100 times more consumers than Croatia itself has. This could be seen as a challenge to compete with products that will be recognized on EU market as safe and traditional. In this order, the approach should be changed to the one called "From Farm to Fork" (*EC*, 2004).

DISCUSSION

Croatia's *integration into the EU* is foreseen as the national and foreign policy priority and for that purpose Croatian Government has proactive approach to co-operate and dialogue with EU, as well as in further preparations for the course of negotiations on EU membership. Perceiving EU membership as a matter of national interest seams to be accepted as such by majority of Croatian society. Screening of the EC regulations, particularly 178/2002, 852/2004, 853/2004, 854/2004 and 882/2004 started through interministerial working groups within CARDS 2002 project during the year 2005. The Food Act ("Official Gazette" No. 117/03; 130/03; 48/04), adopted by the Croatian Parliament in July 2003, has established integrated approach to food and feed safety regulation throughout the food supply chain. This created legal framework for the future approximation of the rules to those of the EU. Adoption of this Act should have created improved organization of inspection and control of food. However, screening in the process of EU negotiations has shown that many parts of the Food Low still have to be adjusted to EC regulation 174/2002.

The *EU negotiations* with Croatia were opened in October 2005 and official screening of food safety legislation chapter started in March 2006. Within more than 30 chapters agriculture, rural development, food safety, veterinary protection and consumers' protection are considered as those of the greater importance. The aim of this process would be complete harmonization of the Croatian legislation with the *acquis communautaire*. However, this will be only the base for parallel process of equal importance, which is strengthening the institutional framework that will be implementing adopted regulations in the future. Croatia intends to conclude the negotiations process by the year 2007 and to reach required level of readiness for full membership in the EU and hopefully become full member country in the year 2009. Preparing for membership in the EU is at the same time an instrument of strengthening Croatian food safety system and embarking on wider-scale globalization processes as well as possible. However, priority will be given to the fact how ready Croatia is going to be for implementing new legislation and proper use of pre-accession foundations. This is considered as more important than the date of accession.

The process of negotiations has improved *coordination between different institutions* in the way of more frequent meetings and discussions about possibilities of improvement of current food safety system. Meetings concerning food safety between Croatian authorities' representatives have much gained in intensivity and are usually coming from DG SANCO missions, CARDS projects, adjusting legislation process etc. Coordination of international projects is under the responsibility of the Ministry of Foreign Affairs and European Integrations (MFAEI) within recently established Directorate for Coordination of Assistance Programmes and Cooperation with the EU. Very often such projects require involvement of different institutions that have the same aim. In order to use projects in best way, it is crucial to form interministerial working groups responsible for each part of the project that involves representatives appointed by ministries or institutions.

One of the most useful directions in order to estimate readiness of Croatia to meet EU food safety standards came *from EC – Health & Consumer Protection Directorate-General – Food and Veterinary Office (DG SANCO) mission in Croatia* in the period from 7 to 11 March 2005 (*EC*, 2005). The Directorate has visited the main food safety authorities in Croatia in order to carry out general assessment of food safety controls and recognize gaps in the system. According to the draft report of the mission, Croatian bodies held general meeting on 12 July 2005 and responded to the Directorate with comments and actions planned or envisaged. The Directorate has estimated Croatian response as acceptable, which proved readiness of all the Croatian authorities to implement EU food safety approach. Beside this report, country profiles on food safety were written (*Antunović*, 2005; *CARDS*, 2006a), which gave good base to set up the action plan.

Croatia has already drafted *Food Safety Strategy* (*CARDS*, 2006b) and is in the phase of discussing it by the representatives of the main institutions involved in food safety. The strategy should revile gaps in the national food control system and facilitate understanding of the needs to change existing food safety system to one that would provide more safety with lower costs. Finishing of national Food Safety Strategy within the CARDS 2002 project (twinning partner Italy) in the year 2006 should give a spin up to all the activities.

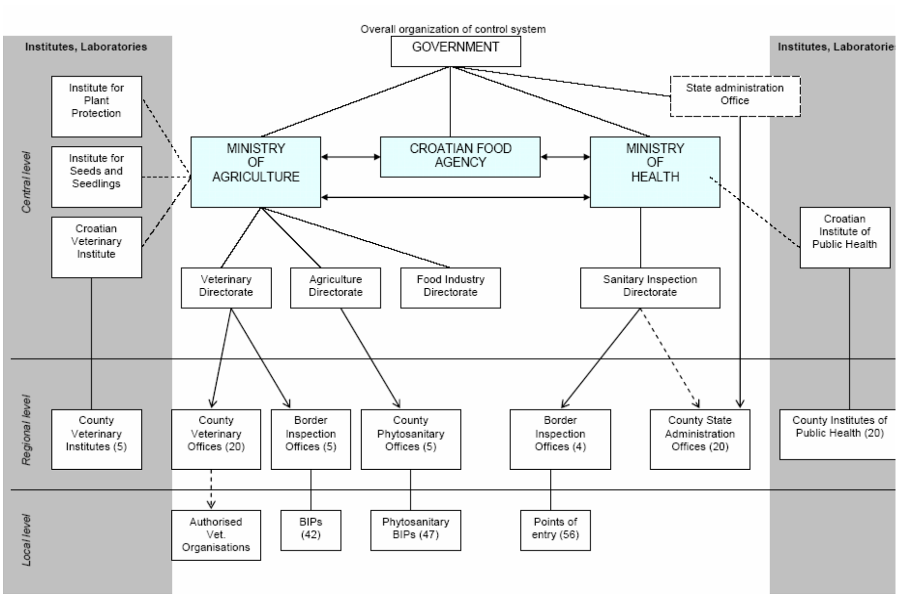
In order to follow high food safety standards, it is necessary to strengthen *connection to EU and world organization* and establish the system of active participation in such organizations. Special emphasis, to ensure compatibility and harmonization among the participating countries and with the EU, should be given to the regional approach. Since a great deal of experience in this field is already present in Central Europe, especially in the last group of accession countries, exchange of experience should be mainly sought from those countries. Additional expertise and technical support should be drawn from the EC directly, or European and world organizations such as FAO, WHO, *Codex Alimentrius*, EFSA and different countries' institutions, depending on level of achievement in each of them. *Codex Alimentarius* is considered as one of the priorities to follow up. Codex Contact point for Croatia was established within Croatian Standards Institute in the year 1994. In April 2006, FAO and WHO organized training in Zagreb and promoted material "Enhancing Participation in Codex Activities" (FAO/WHO, 2005) which raised strong willingness of Croatian authorities to establish National *Codex Alimentarius* Committee.

The main *institutions in Croatia* that are establishing internal and external connections are Ministry of Agriculture, Forestry and Water Management (MAFWM), Ministry of Health and Social Welfare (MHSW), Croatian Food Agency (CFA), as well as other national institutions and laboratories (*Figure 1*).

MAFWM, MHSW and CFA have the overall responsibility for the food chain (plant health, animal health and food safety) in Croatia. The two ministries are preparing necessary rules and regulations related to the area of their responsibilities. In the past there has been some overlapping between their works, but after coming into the force of Food Act ("Official Gazette" No. 117/03; 130/03; 48/04) responsibilities between two ministries were strictly divided. While MAFWM has responsibility for food animal origin, MHSW has responsibility for food of non-animal origin.

Figure 1

Current institutional framework of food safety in Croatia

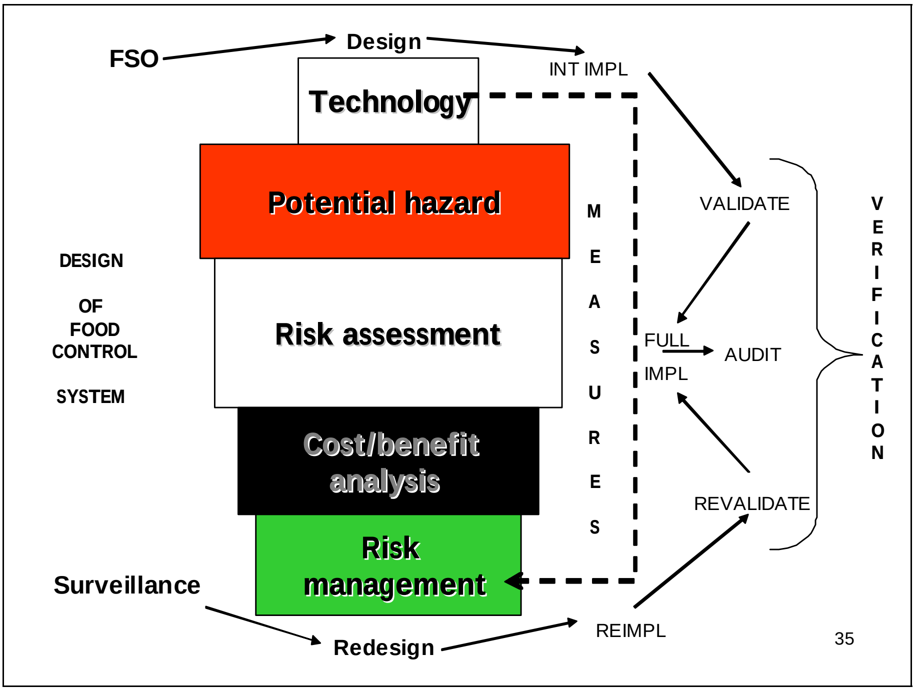


One of the benefits that the Food Act has brought was establishing of the *CFA* as the governmental institution responsible for risk analysis, which started its activity in January 2005 in Osijek and has currently 14 permanent staff, while totally 80 people are involved through the different bodies. All the activities of the CFA are discussed by the Advicing Committee comprising of representatives from ministries, institutes and universities involved in food safety in Croatia, as well as consumers' representatives. Scientific committee is coordinating eight scientific panels, each of them dealing with the following issues respectively: food additives; animal feed; plant health & pesticides; GMOs; dietetic products & nutrition; biological, chemical & physical hazards; contaminants; animal health & welfare. The panels are giving scientific opinion on relevant issues, developing brochures in the area of food safety etc. The organization of CFA is very similar to the structure of the European Food Safety Authority (EFSA).

Within the duties of risk assessment, CFA encourages scientific studies necessary for improving estimation of risks in the area of food and feed safety and establishes unique methodologies for estimation of risks in all phases of production, processing and distribution of food and feed. As the risk assessment procedure is relatively new even in developed countries, there is strong need of education in this field in order to be able to use it according to WTO agreement, as well as to cover national food safety priorities. In this order, CFA is attending and organizing symposia on risk analysis. Recently, CFA has proposed redesign of food control system in Croatia in the way that is shown on *Figure 2*.

Figure 2

Redesign of food control system in Croatia proposed by CFA



According to the Food Act ("Official Gazette" No. 117/03; 130/03; 48/04) the role of the CFA is, beside risk analysis process, in the area of coordination among the relevant bodies in the food safety area. Therefore, the plan was to settle a governmental institution responsible for risk assessment, risk communication and co-ordination of risk management along the whole food chain. However, strong need to separate risk assessment from risk management activities, according to *Codex Alimentarius* recommendations (*FAO/WHO*, 2001), raises new suggestions of revising institutional framework, which is discussed in drafted Food Safety Strategy (*CARDS*, 2006b).

In order to improve *dealing with crisis* in food safety, CFA in cooperation with the MAFWM and MHSW has formed the Interministerial Group for Drafting Crisis Management Plan - general plan for reacting on possible situations of great public concern and Interministerial Crisis Group formed of five highly responsible persons from main institutions. The plan should determine practical procedures crucial for dealing with crisis, including the organization of crisis unit in the competent authorities, equipping it with staff through the application of the principles of transparency and determining the communication strategy between CFA, MAFWM, MHSW, other competent state bodies and institutions, consumers and food business operators.

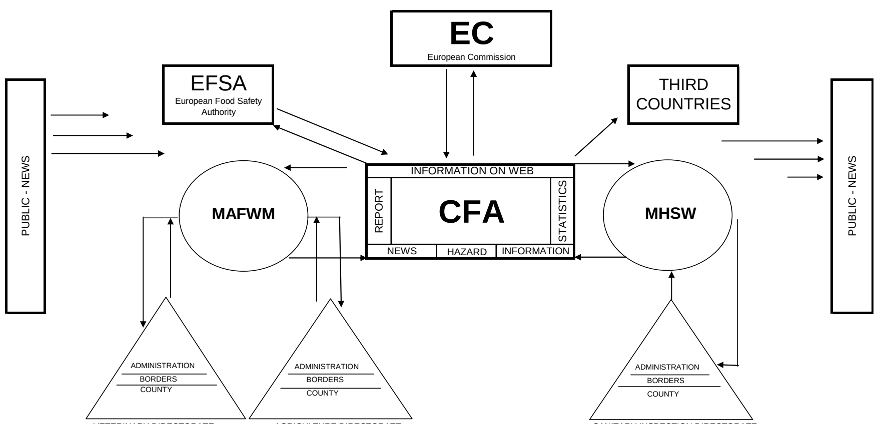
In order to protect consumers' health as fast as possible, CFA has also established emergency phone for coordination among different governmental authorities which is active 24 hours a day. The plan for the next period is to establish video-conferencing room in the CFA office for emergency meetings, especially when scientific advices from scientific panels are needed.

One of the most visible evidence of connecting an accessing country to EU food safety system is certainly *Rapid Alert System for Food and Feed* (RASFF). The RASFF and its development in the Republic of Croatia has been planned as a part of the CARDS 2002 project «Strengthening the Sanitary Inspection» implemented within the MHSW in partnership with the Finnish counterpart. CFA is national RASFF contact point responsible for receiving and forwarding all the information regarding direct food risk for consumers. In the Republic of Croatia, the first RASFF communications were issued in the week 35 of the year 2003 by the Croatian National Institute of Public Health (CNIPH). In the year 2004, the continuity of communication activity went along, while in the year 2005, the newly established CFA took over the role of the RASSFF contact point by taking over and translating communications from the EC on weekly basis, as well as receiving direct communications from the Delegation of the EC in Zagreb and forwarding them to the competent ministries – MAFWM and MHSW. Throughout the year 2005, CFA has received the total number of 23 communications from the Delegation of the EC in Zagreb, out of which 10 communications were related to the food originating from Croatia.

The CFA has started implementing the pilot project for setting up RASFF in Croatia in 2006 (*Figure 3*) with the aim to achieve readiness to connect to EC in year 2008. The project will be moved forward in several stages, whereas the first stage involves setting up of the internal system for Croatia along with delivering general plan for crisis management and guideline for recall or withdrawal of products from the market. In the course of the years 2007 and 2008, an exhaustive IT system covering all competent inspection services (central, border and local) in the area of food and feed is planned to be implemented.

Figure 3

Proposal model for RASFF in Croatia



The RASFF pilot project comprises following steps:

a) Description of the framework and timeline for the program completion;
b) Defining role and capacity of the participating institutions;
c) Establishing procedures and protocols;
d) Training and necessary information;
e) Audit procedure.

As the part of the project, following activities have been implemented up to now:

- Interministerial group for RASFF has been appointed – contact persons (and their deputies) in CFA, MAFWM (Phytosanitary Office, Veterinary Directorate, and Agriculture Directorate) and MHSW (Sanitary Inspection Directorate);
- Contact points of RASFF system have been appointed – veterinary and sanitary inspectors in the MAFWM and MHSW;
- Development of the Treatment Protocol has been in progress – the Treatment Protocol shall include the list of contact persons in order to provide efficient, documented and immediate information to the superintended contact points on each alert.

The Croatian RASFF pilot project is currently involving 12 teams within Agriculture Directorate and Veterinary Directorate of the MAFWM and Sanitary Inspection Directorate of the MHSW. The project, commencing with its work in the first week of 2006, has produced weekly reports and communications, out of which alerts and information were published on official CFA web site (www.hah.hr). Following activities are scheduled to be carried out within the same project framework in the next period:

- Development of communication system - forms and forms attachments are prepared according to those currently used in the EU as the standard RASFF forms (form A contains all data on risks, product, producer, distributor and retail, while form B contains all the data on steps taken to minimize the risk);
- Caring out the training of staff involved in the process related to: data protocols for communications on risks, information notices and EC notices, ways of presenting information to the public, traceability, recall, administrative and other proceedings;
- System development - development of the strategy for providing information to the public – provision and/or selection of relevant information for the public and industry by the means of web pages, press and direct contacts with industry and consumers' associations;
- Once the operation system is put in place it should be transferred into information technology (IT). This involves investment into IT and implies participation of all the inspection offices operating within various competent bodies, as well as development of the central data base with an Internet/Intranet link for all the authorized users.

The pilot project main goal is RASFF trial implementation in the Republic of Croatia, while expected results include the operational network, detection of possible obstacles and omissions, their removal and obtaining the pilot annual report for the year 2006. In case that setting up of the RASFF system becomes fully operational, in the year 2007 the project would be extended to all the participants and finally it would become part of the EU RASFF network.

Beside RASFF, establishing system of gathering reliable data represents one of the most important steps in order to be able to use data for risk assessment. Development of *Central Information System (CIS)* in the area of food safety in Croatia is a part of the World Bank project/loan «Agriculture Acquis Cohesion Project» implemented within the MAFWM. Currently, laboratories involved in food safety in Croatia have an obligation for giving annual reports to CFA, which collects all the data necessary for risk assessment. Due to very different reports obtained, Advising Committee of CFA has approved the unique data sheet that is now in usage. The plan for the year 2006 is to set up the architecture of IT system, and thereafter, to issue a call for procuring equipment. Development and setting up of the system for delivering the sector-specific software has been scheduled for the year 2007. Fully developed system, including all the pertaining software and statistical data processing, together with the availability of complementary integration with the identical systems in the EU is timetabled for the year 2008.

Data are currently being collected from the total number of 54 laboratories that perform *food and feed safety analysis* in Croatia. The ministries are approving them for work and accreditation is being done by recently established Croatian Accreditation Agency (CAA). Beside governmental laboratories, there are eight private laboratories in statutory testing network and also ten laboratories within the faculties, which are opened for public services. Seven laboratories are accredited according the HRN EN ISO EC 17025. Accreditation is considered as priority for labs to make them able to provide official control from the moment of EU accession.

Food safety in Croatia is monitored continually in production and trade, as well as when food is imported. The most frequent reasons for declaring food unsafe among the microbiological parameters are increased number of *Salmonella, Enterobacteriae, Staphylococcus* and sometimes *E. coli.* Among chemical parameters, one of the most frequent reasons is usage of impermissible additives in particular food, as well as increased quantity of additives in certain cases. Presence of toxic metals, pesticides and other contaminants in impermissible amounts is rare cause of finding food being unsafe on the Croatian market.

In order to be able to interpret data, laboratorial results always have to be considered together with *epidemiological data*, as well as with *inspections' data*. Microbiological contamination of food is the most reported cause of consumers' poisoning in Croatia, as well as in the whole world. Due to the changes in lifestyle and dietary habits of people, globalization of food supply, etc., this problem gets bigger all the time and incidences of alimentary infections and intoxications are becoming more frequent. Usually, causes of epidemics are salmonellas and staphylococcus toxin, as well as trichinella among parasites. Over the recent years, the number of reported outbreaks has been within the range of up to one hundred a year (73 on average).

Human resources employed in the area of food safety, animal health, animal welfare and plant health in Croatia are estimated to approximately 2.000 professional staff employed. Human resources development plan in the area of food safety should be carried out parallel with harmonization of legislation and should include chain "education of educators" process.

Trainings of various control officials on inspection and sampling in general and on Hazard Analysis and Critical Control Points *(HACCP)* are of the greatest importance in order to fulfill new food safety approach requirements. Giving more responsibilities to producers by implementing HACCP principles requires changes in inspectors' attitude to food control by shifting focuses to establishments' control.

Consumers' protection, as natural persons acting on the market for furtherance of their nonprofessional interests, is recognized in Croatia as an area that requires special attention. Providing information to the public on food safety issues and being transparent is one of the priorities of CFA work in order to become trustful source of informations and reliable base for making decisions by risk managers. CFA official website (www.hah.hr) is daily updated with news, RASFF informations, invitations to seminars, brochures etc. Furthermore, the internet forum, active on this page, is opportunity for consumers to discuss about food safety issues.

Every two years the Parliament adopts the National Consumer Protection Programme, defining priority tasks in the area of consumer protection to be financed from the state budget. Once a year, ahead of the World Consumer Day, the Government reports to the Parliament on the results of the consumer protection policy, as defined in the national programme for preceding year. The Government appoints the Consumer Protection Council, consisting of various representatives, whose activities are considered public.

Consumer protection associations established by consumers are non-profit organizations tasked to promote and protect consumers' rights. The work of consumers' associations includes in particular: consumers' information; product testing at licensed laboratories; comparative product testing; assisting consumers in voicing their grievances before the trader; coming forward with comments and proposals on regulations relevant to consumer protection being in the process of adoption; taking legal actions envisaged by the legislation. From recently, financial support to non-governmental consumer associations is provided by the EC through the project "Capacity Building in the Area of Consumer Protection", which includes IT equipment and technical assistance required for their work. State institutions and public offices involved in food safety inform public about influences on people's health, and according to that they undertake adequate steps, decrease or eliminate risk. CFA, therein, communicates through TV, newspapers, web page, consumers' forum and CID, i.e. free phone.

CONCLUSIONS

Taking in account everything above mentioned, following steps are considered as the priorities in order to establish new food safety approach in Croatia as an EU accessing country:

Food safety policy:

- revising institutional framework;
- finishing National Strategy on Food Safety;
- developing General Program for Crisis Management in food safety area;
- writing and appliance of with EU adjusted food safety regulations;
- promoting Codex Alimentarius standards and establishing Croatian Codex Committee.

Communication:

- implementing RASFF and active role within it by connecting CFA to DG SANCO;
- connecting all the institutions and approved laboratories that are related to food safety to CFA (building Central Information System).

Food control:

- education and reorganization of inspections on specific issues;
- upgrading network of accredited laboratories according to EU requirements.

Risk analyses approach:

- education on risk analyses process;
- developing coordinative risk management body - decisions being made based on risk assessment results;

- providing risk assessment based on reliable data;
- improving system of gaining data needed for risk assessment;
- developing scientific studies necessary for filling gaps in risk assessments.

Producers and distributers:

- introducing HACCP system in producing and distributing units;
- developing guidelines for GMP and HACCP and application of those systems;
- education of inspections on HACCP system;
- improving traceability through introducing system „one step back – one step forward";

Consumers:

- building – up consumers' confidence – developing Public Communication Strategy – improving transparency;
- promoting healthy nutrition.

Cooperation:

- connecting with the EC and EFSA;
- connecting to similar institutions in Europe and World (regional and global cooperation);
- cooperation with FAO/WHO and other European and World associations.

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Genetic Traceability of livestock products

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ABSTRACT

Aim of this review was to describe the novel approaches on genetic traceability of livestock products. The term traceability, regarding the livestock production sector, means the ability to keep under unfailing control the products origin and animals identity along all passages of the food chain, from farm to fork. In this way it represents a warranty both for the consumers and the producer and it will permit to know where, who and how a product has been produced. It is clear that traceability could be an important tool in order to preserve and to turn to account the livestock products, especially for typical ones. Different kinds of traceability (conventional, aromatic, geographic and genetic) are discussed in order to explain the principles on which they are based and their possible applications. Genetic traceability is based on DNA identification technology through the use of molecular markers. The genetic traceability might be used at four different levels: individual, cohort/group, breed and species. Regarding genetic traceability, the effective discrimination at level of unique animal identification depends on reducing the probability to find two individual sharing, by chance, the same genetic profile to an acceptable low threshold. For example in a standard proceeding even a two locus test with polymorphic markers as microsatellites (one in sixty-four chance of error, i.e. accidental match) might be sufficient to reach a verdict, but for a forensic case, eight loci (one in 16.8 million chance of error) might be sufficient to reach a verdict. The effective discrimination from the point of animals group (herd, breed or species) identification is based on two different approaches: deterministic and probabilistic. Deterministic approach is based on the analyses of neutral molecular markers specific for each breed and/or genes with different allelic forms fixed within breed as genes affecting coat colour. Probabilistic approaches are based on two methods, the first using the allelic frequencies typical of each group (herd, breed or species) while the second using genetic distances among groups. In conclusion, this review, showed as the novel approaches on genetic traceability of livestock products is an available method even if it should be improved in terms of cost reduction for single sample, work effort, reproducibility and accuracy of results. At the time genetic traceability is an important method for origin identification of livestock products and a tool for guarantee conventional traceability system as routine method for food safety.
(Keywords: genetic traceability, DNA identification, livestock products, food safety)

INTRODUCTION

Currently traceability of food production is a priority on the international agenda of various global organisations such as the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO). It estimated that the world population exceeds 6 billion people, who in turn support, and are supported, by some 17 billion poultry and nearly 4.5 billion livestock, according to the latest FAO statistics. With the markets globalisation agriculture has become "anonymous", alimentary raw materials are produced where they are cheaper and consumers do not know neither about food origin nor the producing companies (*McKean*, 2001). In the last few years the discussion on the identification and registration of GMOs (genetically modified organism), between the EU and the USA, contributed to increase the traceability requirements and transparency in food chains. Labelling of GMOs is obligatory in the USA only if the product differs essentially from the "original", e.g. if the nutritional value differs, or if the product contains an allergen that it is not present in the original. The EU demands that all GMO products, with a GMO contamination of >0.9%, must be labelled as such. Moreover, the social and economical changes in developing countries have focused more attention on the consumer point of view on the origin and food safety of animal products, particularly after negative events such as BSE (bovine spongiform encephalopathy), chicken dioxin contamination and the recent avian influenza that are only some examples which reported attention on animal products traceability and suggested EU legislators to introduce specific new laws (EU regulation 178/2002) in the food safety sector. Therefore, the traceability become an answer of the producers to the consumers that need more public trust in term of safety and quality for food of animal origin. Moreover, in the last years, the valorisation of traditional and protected products, whose Italy is leader in Europe and in the world with 145 PDO and PGI and more than 1400 traditional products (included in the list filled according to the DM 350/1999), is an interesting way for the development of livestock systems located in less competitive areas but that still have to face the market competition. These products embody typical added values represented by tradition, high quality and, sometimes, a close link with animal breeds at risk of extinction resource, allowing their selling at a higher price. Traceability could be a method to safeguard and guarantee the origin of these products as well. Traceability is also considered a fundamental tool in the White Book written by the European Union Commission that represents a basic element of European Regulation concerning responsibility for damages due to "defective" product. Therefore, traceability methods can become an effective way to develop new relations between the world of production and the world of consume.

TRACEABILITY SYSTEMS

The basic characteristics of traceability systems are similar, requiring product identification, product tracking and the maintenance of information relating to the product and its movement. Yet there remains a lack of clear consensus to how traceability is achieved in practice. The ISO 8402 standard defines traceability as "the capacity for establishing a product's origin process history, use and provenance by reference to written records' (*ISO*, 1994). However, like other traceability definitions, ISO 8402 does not define which parameters have to be measured or how history or origin should be determined. In a report on traceability systems, *Golan et al.* (2004) underline three key parameters that can be used to characterise traceability systems, as follows: 1) the breadth; 2) the depth and 3) the precision of the system. The breadth of the traceability system is due to the amount of information recorded (e.g. feed regime, pedigree information or details of animal's veterinary care), the depth of the system is how far back or forward the system tracks (to a grain elevator, farm or field); in many cases, the depth of a traceability system is determined by its breadth or attributes of interest. The precision of the system is the degree of assurance with which the tracing system can pinpoint the movement of a particular product, and is described with reference to an acceptable error rate, or what would happen if there were mistakes in tracking the product.

An important key of any traceability system is the ability to clearly identify what it has to be traced. Ideally the product identifier should uniquely guarantee that the identification of the unit or batch is sure (fraud proof), permanent, retaining identity throughout the product life-cycle, simple to read and capture identifying data and not hinder its host.

In practice no single identification system is likely to meet all these requirements, for this reason the choice of method(s) will ultimately be determined by the specific needs of the supply chain in question.

In general terms, at present, there are three different types of traceability available: a) conventional, based on labelling of food (the present law on labelling of beef meat is a clear example); b) aromatic or geographical, based on identification of specific aromatic compounds and on the presence of specific micro organisms in typical products of specific areas; and c) genetic, based on DNA analysis.

CONVENTIONAL TRACEABILITY METHOD

The conventional traceability method is based on external identifiers that are applied to the animal/product and can become unwieldy to implement in more complex supply chains. External identifiers types include both manual methods such as paper labels, in brands (tattoos) and plastic ear tags, and electronic methods such as Radio Frequency Identification (RFID) tags and inject able microchips. The advantage of these approaches lies in they ability to encode different types of information (barcode symbologies can contain information relating to the product and its process history), and the relative ease with which the data can be read in real-time, facilitating the use of electronic identifiers for monitoring animal movements. For example, maintaining individual animal traceability within a meat processing environment could lead to a proliferation of labels to track all the pieces of an animal post slaughter. But possible of greater concern is the fact that external identifiers may become separated from the product through tag/label loss or removal, and are susceptible to fraud. Within the meat processing sector, an EU report found that through the use of a conventional meat labelling system "in many member states serious deficiencies were found in the ability to trace back meat from retail and distribution centres, even to the preceding stage of the production chain" (*European Commission*, 2003).

AROMATIC AND GEOGRAPHICAL TRACEABILITY

The aroma concept is based on the sensory characteristics tied to the presence of volatile substances (of low molecular weight) and not volatile substances (of higher molecular weight) present in animal products (milk, meat, cheese etc.). Some factors that modify the aroma are the heat treatments before the packaging, the cycle oestral of the animal and the type of feeding; it seems in fact that the milk ones produced in mountain area has a more intense aroma which depends on the diet of the bovine to the pasture. In order to verify the effect of herd management influences on aromatic property of milk, *Bailoni* and *Mantovani* (2000), compared the value of some compounds determining the aroma in dairy herds of the plan fed with traditional feeding or unified with dairy herds fed with fresh mountain forage; in the second group they detected a significant increment on aldehydes and ketones.

The geographic traceability instead is based on the determination of the geographic origin studying the bacterial composition of natural serum cultures for the production of cheese products. In fact such coltures could introduce some differences in the products microflora due to the various area of production, therefore they would allow to distinguish cheeses, even of the same variety, but produced in different geographic areas. An example has been put to point for the mozzarella in the area of Caserta and Salerno (*Mauriello et al*., 2003).

GENETIC TRACEABILITY

Genetic traceability system might be considered as a biometric labelling system that incorporate biological data and cannot easily be faked, altered or appropriated. This biotechnology includes DNA profiling, retinal scanning and nose printing. Moreover, to being less prone to error or fraud, these biometric labelling methods are permanents, covering the life history of the animal, and in the case of DNA the full product life history. The basic principle underlying DNA-based traceability is that each animal is genetically unique (except in the case of identical twins or clones) and that the animal’s own DNA code can be used to identify it and its products as its own label. *Jeffreys et al*. (1985) discovered that when DNA is digested with specific enzymes, the pattern of resultant DNA fragments, resolved by gel-electrophoresis, is specific to the individual. This process became widely-known as DNA fingerprinting. This technology was initially applied in forensic studies and proved an extremely powerful source of evidence in legal cases. However DNA fingerprinting required a relatively large amount of high quality source DNA and this was not always available, particularly in forensic cases. The development of another process by *Mullis et al*., (1986), and the application of this process to a particular type of DNA sequence led, in 1989, to the development of current DNA identification technology. The basic principle was to generate, in a test tube, large quantities of specific target DNA sequences, where the sequences are specifies by a pair of short (around 20 bp) artificial DNA primers. This process, which is known as the polymerase chain reaction (PCR), has become the pillar (foundation) of modern molecular genetics. However, the PCR alone is not sufficient to allow individual identification. It is necessary to find sequences of DNA that vary among individuals. In 1989, the PCR process was first applied to a type of variable DNA sequence called simple tandem repeats (STR) or microsatellites, and this led to present day genetic identification more generally known as DNA profiling.

LEVELS OF GENETIC TRACEABILITY

The genetic traceability might be used at four different levels: individual, cohort/group, breed, species.

Individual traceability is a food safety control able to guarantee the consumers from frauds, it is of a great importance in the beef sector as control of the conventional labelling system (*Portetelle et al*., 2000; *Sancristobal-Gaudy et al*., 2000; *Cunningham et al*., 2001; *Arana et al*., 2002). However, for the milk-cheese chain and other animal products made by groups of animals the individual traceability is not directly useful but only to reconstitute the origin group or cohort. This system might be very interesting for cheese and other products by multi-individuals (*Cocucci et al*., 2002) in this case the right term is herd or cohort traceability.

Breed and species traceability can verify with scientific and objective methods the origin of animal products (*Milanesi et al*., 2003; *Ciampolini et al*., 2006; *Ovilo et al*., 2000; *De Marchi et al*., 2003); it is of particular interest for products such as cheese and processed meat, that are strictly linked with only one breed or species (*Alves et al*., 2002).

In this case traceability is very useful for quality certification as the European PDO and PGI label that can support the economic development of marginal areas increasing the add value of typical or niche products often linked to animal genetic resources under conservation schemes (*Gandini* e *Oldenbroek*, 1999; *Milanesi et al*., 2003). For these reasons breed traceability is an important topic of research in Mediterranean countries (Italy, Spain and France) where it could be found a high number of typical products (*Pancaldi et al*., 2005) that often are mono-breed. In Italy there are some famous PDO "monobreed" cheeses such as the Fontina Valdostana (obtained with milk of the Valdostana cattle breed) and the Parmigiano Reggiano obtained only by the Reggiana cattle breed. There are also other examples of typical monobreed cheeses but, in this case, they are not yet protected by the European label (the Spressa and Morlacco produced by the Rendena and Burlina cattle breeds, respectively). In the beef sector there is also an example of PGI labels (since 1998) of the "Vitellone Bianco dell'Appenino Centrale" made by the Chianina, Marchigiana, Romagnola, Maremmana and Podolica cattle breeds. The pig sector based on ham production it is also interested on traceability method not firstly for identify the breed of origin but for exclude the use of specific pig breeds such as the Pietrain and Belgium Landrace that produce meat of low quality characteristics.

Following the classification proposed by *Ajmone-Marsan et al*. (2004), the studies on breed traceability were based on two different approaches: a) deterministic approach and b) probabilistic approach. The deterministic approach is based on the analyses of neutral molecular markers specific for each breed (*Negrini et al*., 2003; *Alves et al*., 2001) and/or genes with different allelic forms fixed within breed (Miladnesi *et al*., 2003). The major researches on this approach are based on genes affecting coat colour (*Crepaldi et al*., 2003; *Russo* and *Fontanesi*, 2004; *Maudet et al*., 2002; *Carriòn et al*., 2003; *Alderson et al*., 2003; *Fernandez et al*., 2004). The probabilistic approach is based on two methods, the first one using the allelic frequencies typical of each breed, and the second one using the genetic distances among breeds (*Milanesi et al*., 2003).

POWER OF DISCRIMINATION (NON È MEGLIO LEVEL)

Generally, for a traceability system, the basic question is whether two samples are the same or different. The answer, in the case of a genetic traceability method, is a matter of probability. Indeed, if we assume for an individual animal, ten individual simple tandem repeats (STR) loci and each STR have four alleles, for each STR the animal possess two of the four possible alleles, one inherited from the father and one from the mother. The two alleles, together are refereed to as a genotype. Therefore, ten genotypes represent the twenty alleles in the ten loci STR profile for this animal. The probability that any other animal shares, by chance, this exact combination of genotypes is low. For example if the frequency of each of the four alleles for the ten STR is assumed to be 25%, the cumulative probability (%) of a chance match is 9×10^{-8} or $0.125^{n}\times100$, where n is the number of loci considered.

Hence, the effective discrimination to the point of unique animal identification depends on reducing this probability to an acceptably low level. The basic for declaring a match depends on the purpose to which the information is to be put. In a standard proceeding, for example, even a two locus test (one over sixty-four chance of error, i.e. accidental match) might be sufficient to reach a verdict, in a forensic case, eight loci (one in 16.8 million chance of error) might be sufficient to reach a verdict.

A significant new development in the field of molecular genetic identification is currently underway. A new class of DNA markers called single nucleotide polymorphisms (SNPs) has been researched. As the name indicates, a SNPs concerns genetic variation at the lowest possible level that is at a single base or nucleotide. As a result, the amount of genetic variation in each such unit is limited. In contrast to microsatellites with numerous alleles, SNPs have only two alleles. This makes SNP analysis highly amenable to full automation. A possible limitation is that a larger number must be tested in order to achieve satisfactory power of discrimination.

CONCLUSIONS

The livestock production sector is addressed towards a future in which the herds will be specialized in advantaged areas, instead in the disadvantaged areas will be necessary that the companies are adapted to have a multifunctional role not being in a position to being competitive in terms of production. In both cases the companies will have to guarantee a sustainable development and to find a balanced combination between new technologies and protection of the typical products giving always greater importance to the alimentary emergency and food safety using traceability systems.

Different kinds of traceability systems (conventional, aromatic, geographic and genetic) are available for field application. At present, genetic traceability, based on technology of DNA identification trough the use of molecular markers, seem to be very important for guarantee conventional traceability system as routine method for food safety.

In the next future, the reduction of costs and the organisation system for recording and stocking organic and DNA samples will permit an application of genetic traceability as more routine method even if the reproducibility and repeatability of these molecular methods should be studied.

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Meat and milk quality and safety: past and future trends in Slovenia

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ABSTRACT

In Slovenia, meat production has stabilised in the last years. The important increase in meat production was noticed in sheep and goats. The quality of slaughtered cattle, estimated on the basis of noted conformation and fatness, has worsened in the last decade, mainly due to changes in the structure of cattle population. On the contrary, large improvement in quality was realized in pigs. Lean meat content increased around 6%. Sheep and goat meat production rapidly increased in the last 15 years (500%). Poultry meat producers have focused mainly to safe, healthy and functional products to satisfy the consumers' needs. Milk production was limited by negotiated quotas and reached 635 million litres in the year 2005. Protein and fat content did not change in the last seven years, but the quality of milk regarding the number of total bacterial and somatic cell count greatly improved.
(Keywords: meat, milk, production, quality, Slovenia)

INTRODUCTION

After the independence the Slovenian agriculture has experienced great alterations. Development Strategy of Slovene Agriculture was passed by the Slovene parliament in 1993 and agriculture development was orientated to market economy that considers environmental and social conditions, and sustainable market economy. Farmers are aware that they will be successful in the EU if they adapt to the new conditions as soon and as best possible. It became obvious, that at least as important as the quantity of produced food will be the quality of produced foods. So, a lot of efforts were invested to assure and improve the quality of animal products.

The aim of present work was to analyse trends in the quality of produced meat and milk in Slovenia in the last decade and to present our future expectations.

MEAT PRODUCTION

Meat production in Slovenia in the last 10 years can be seen in *Table 1*. Total meat production was relatively stable and reached around 170 thousand tons. After a small drop between 1995 and 2000, meat production increased for almost 10 thousand tons. The quantity of meat from animals slaughtered in slaughterhouses was increasing up to 2003 and then it decreased in the year 2004. Veal and beef production reached 45 thousand tons in the year 2004. Pig meat production was estimated to 71 thousand tons. Poultry meat production has stabilised at around 53 thousand tons. The greatest increase has been achieved in sheep and goats, where the production of sheep meat has more than tripled and goat meat has doubled in the last 10 years, although it is still very low.

Table 1

Meat production in Slovenia (in 1000 t) (*Statistical yearbook*, 2005)

	1995	2000	2001	2002	2003	2004
Gross indigenous production[1]	160.4	151.5	173	159.6	176.7	169.1
Meat from slaughter in slaughterhouses[1]	119.6	124.7	131.6	129.0	136.5	126.7
Cattle						
Gross indigenous production	43.0	39.3	48.0	44.6	49.6	45.0
Meat from slaughter in slaughterhouses	33.0	34.6	39.2	40.5	43.1	40.1
Pig						
Gross indigenous production	65.1	58.1	66.4	61.9	70.3	71.2
Meat from slaughter in slaughterhouses	36.5	38	35.8	37.1	37.3	34.6
Poultry						
Gross indigenous production	52.3	54.1	58.6	53.1	56.8	52.9
Meat from slaughter in slaughterhouses	50.1	52.1	56.6	51.4	56.1	52.3
Sheep						
Total indigenous meat production (live weight)	0.9	2.7	2.4	2.9	2.5	3.3
Goats						
Total indigenous meat production (live weight)	0.3	0.7	0.4	0.6	0.7	0.6

[1]Mutton, lamb, goats and horse meat is not included.

VEAL AND BEEF PRODUCTION

In the year 1994 the carcass classification of slaughtered cattle was introduced according to the EUROP system. (*Pravilnik o ocenjevanju in razvrscanju govejih trupov in polovic na klavni liniji*, 1994). It has foreseen six categories of slaughtered cattle (calves under six month of age and 160 kg carcass weight, young bulls under 24 months, old bulls over 24 months, heifers, steers and cows). Later on some changes have been introduced, so the age and weight of the slaughtered calves have been increased to 8 months and 185 kg (*Pravilnik o ocenjevanju in razvrscanju govejih trupov in polovic na klavni liniji,* 2001, 2004). The percentage of slaughtered cattle in different category is presented in *Table 2*. After the increase of classified cattle up to 2003, it diminished slightly in the years 2004 and 2005. The percentage of slaughtered calves presented around one quarter of all slaughtered cattle. For beef production the most important categories were bulls, cows, heifers and steers. The age of slaughtered animals was subjectively defined by the ossification of vertebra up to the year 2001. After that the age was defined on the basis of birth date provided by SIR (Cattle Identification Service of Slovenia). Consequently, bulls that were slightly older than 24 months and were before classified as young bulls were then classified into the category of old bulls. So the percentage of bulls older than 24 months increased dramatically in the year 2002. The percentage of slaughtered cows increased due to the strict veterinary legislation and complete control through animal identification service, presenting now around one quarter of slaughtered cattle. Heifers presented slightly more than 10% of slaughter cattle, while the number and percentage of slaughtered steers was negligible.

To evaluate the carcass quality of slaughtered cattle, we collected data from slaughterhouses which have automatic data collection. In year 1997 seven slaughterhouses were included in data collection, up to the year 2005 the number had been increased to eleven. At the beginning of classification the percentage of included animals from total slaughtered and classified in Slovenia was around 50% for each category. In 2005 it increased to 83.6%.

In *Table 3* the number and carcass weight of slaughtered cattle is shown. The number of classified calves was increasing in the studied period up to 2004 and diminished slightly after that. The percentage of calves included into the valuation in comparison to all slaughtered calves increased from 48% in the year 1999 to 81% in the year 2005. The average carcass weight of calves through the last nine years was 75 kg. Though it increased for 15 kg, it is with 82 kg still very low in comparison with Austria, for example (*Zaufaly et al.,* 2005). The average carcass weight of young bulls was 346 kg and slightly varied in the studied years. It was increasing up to 2002, and started to diminish after that. Up to the same year the carcass of old bulls was more than 50 kg heavier than that of young bulls. Afterwards the number of slaughtered old bulls increased and the carcass weight diminished, so the difference came to only about 20 kg (*Table 4*). Carcass weight of heifers diminished slightly in the period from 1997 to 2005, and was on average for almost 90 kg lighter than young bulls. Carcass weight of cows was with 294 kg for 34 kg heavier compared to heifers.

The best conformation was estimated for the old and young bulls, which on average achieved almost 3.5. Slightly worse conformation was noted for heifers and the lowest for cows and calves. The best conformation was noted in the year 1998 and 1999. Afterwards the conformation notes decreased dramatically in all categories (the least in young bulls for two thirds of conformation class and the most in old bull for almost one conformation class). At this time the cattle population in Slovenia has continuously changed due to increased percentage of dairy cows and crossbreds between Simmental and Montbeliard and Red Holstein. The second reason for those changes could be also the criterion for conformation estimation which had been sharpened in that period.

The highest carcass fatness was noted for heifers (3.16) and the lowest for calves (2.32). Carcass fatness of old bulls, young bulls and cows was on average very similar. In the studied period carcass fatness diminished in all categories, most in calves and least and less in other three categories (*Table 5*).

Table 2

The number of all classified cattle and percentage of different categories in Slovenian slaughterhouses from 1998 to 2005

	Number of all classified animals	Calves	Young bulls*	Old bulls*	Heifers	Steers	Cows
1998**	123.560	19.3	65.6	0.6	-	-	14.5
1999**	128.022	21.3	64.1	0.5	-	-	14.1
2000**	126.898	22.5	61.7	0.9	-	-	14.9
2001	143.473	17.0	49.9	2.6	11.5	0.2	18.8
2002	157.005	18.0	35.8	10.7	12.1	0.3	23.1
2003	165.763	18.3	36.6	7.9	12.7	0.4	24.1
2004	153.799	20.1	38.7	8.1	12.1	0.3	20.7
2005	140.692	19.5	35.5	14.5	11.5	0.4	18.6

* young bulls – bulls younger than 24 months, old bulls – bulls older than 24 months; ** in the year 1998, 1999 and 2000 the percentage of young bulls includes also heifers and steers.

Table 3

Number and carcass weight of slaughtered cattle in slaughterhouses with automatic data collection during the period from 1997 to 2005 in Slovenia

Year	Calves		Young bulls (under 24 m.)		Old bulls (over 24 m.)		Heifers		Cows	
	N	kg	N	kg	N	kg	N	kg	N	kg
1997	6721	67	32854	340	224	409	10660	262	9702	282
1998	9875	67	33090	348	216	389	9921	265	8414	292
1999	13188	68	32516	350	218	409	9808	264	8149	298
2000	14991	69	36220	353	491	420	10718	264	11571	290
2001	10230	82	46173	354	2365	409	11997	269	16755	289
2002	13232	80	35483	343	9988	363	12876	261	32347	313
2003	22828	78	47886	340	10049	357	16234	251	39990	308
2004	23244	79	47933	341	9710	360	15377	251	25442	291
2005	22330	82	42921	342	16370	365	13972	255	21559	283
Σ/Avg.	136639	75	355076	346	49631	387	111563	260	173929	294

Table 4

Carcass conformation of slaughtered cattle in slaughterhouses with automatic data collection during the period from 1997 to 2005 in Slovenia

Year	Calves	Young bulls (under 24 m.)	Old bulls (over 24 m.)	Heifers	Cows
1997	2.85	3.62	3.78	3.37	2.94
1998	2.90	3.65	3.52	3.44	3.00
1999	3.10	3.62	3.71	3.46	3.01
2000	2.98	3.58	3.88	3.41	2.96
2001	2.79	3.51	3.77	3.35	2.97
2002	2.60	3.44	3.37	3.32	2.93
2003	2.68	3.30	3.19	3.12	2.69
2004	2.49	3.17	3.07	2.73	2.50
2005	2.35	2.98	2.92	2.78	2.22
Σ/Avg.	2.75	3.43	3.47	3.22	2.80

(E=5, U=4, R=3, O=2, P=1)

On the basis of conformation and fatness notes, carcasses are classified into 6 payment classes, 1 beeing the best and 6 the worst. As we can see in *Table 6* the best payment class was attained by young and old bulls, heifers, calves and cows. Due to worse conformation payment class also worsen.

Carcass conformation is defined mainly by animal genotype. So it can be improved through selection and introduction of new genotypes. The most effective way is industrial crossbreeding with beef breeds. Fattening technologies will have to adapt to achieve optimal fatness at slaughter in different categories. Basic concepts of animal growth regulation and control will have to be considered (*Hossner*, 2005).

Table 5

Carcass fatness of slaughtered cattle in slaughterhouses with automatic data collection during the period from 1997 to 2005 in Slovenia

Year	Calves	Young bulls (under 24 m.)	Old bulls (over 24 m.)	Heifers	Cows
1997	2.62	2.89	3.14	3.22	2.79
1998	2.57	2.88	3.13	3.25	2.79
1999	2.60	2.89	3.00	3.24	2.85
2000	2.51	2.88	3.09	3.24	2.77
2001	2.29	2.88	3.03	3.30	2.84
2002	2.14	2.86	2.74	3.20	2.78
2003	2.06	2.75	2.64	3.04	2.67
2004	2.03	2.66	2.54	2.94	2.60
2005	2.03	2.65	2.55	2.97	2.57
Σ/Avg.	2.32	2.82	2.87	3.16	2.81

Table 6

Payment class of slaughtered cattle in slaughterhouses with automatic data collection during the period from 1997 to 2005 in Slovenia

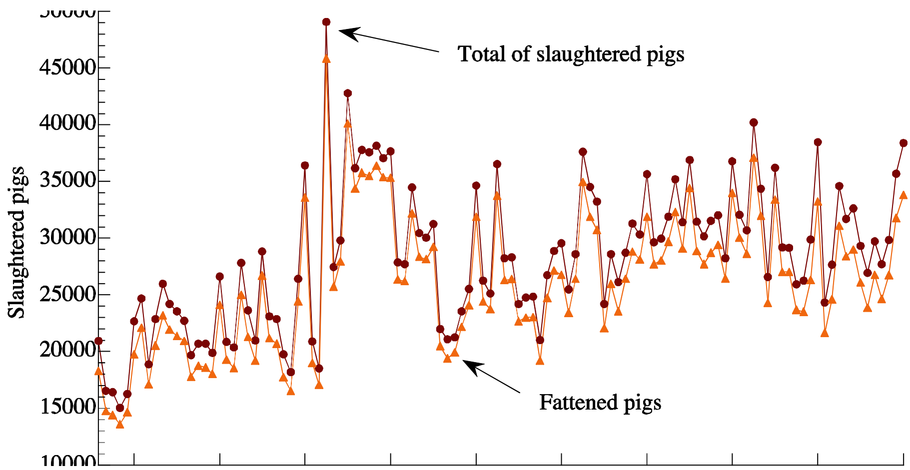
Year	Calves	Young bulls (under 24 m.)	Old bulls (over 24 m.)	Heifers	Cows
1997	3.18	2.40	2.43	2.94	3.34
1998	3.13	2.38	2.69	2.89	3.30
1999	2.92	2.42	2.46	2.86	3.33
2000	3.04	2.47	2.29	2.90	3.36
2001	3.27	2.56	2.38	3.04	3.34
2002	3.39	2.62	2.67	3.00	3.37
2003	3.40	2.75	2.85	3.14	3.66
2004	3.61	2.98	2.97	3.26	3.82
2005	3.76	3.09	3.13	3.45	4.17
Σ/Avg.	3.30	2.63	2.65	3.05	3.52

PIG MEAT PRODUCTION

The improvement in pig carcass quality has been estimated on the base of carcass grading in slaughter houses. The total production comes to around twice the registered number due to relatively high proportion of slaughter for home use and slaughter in small slaughter houses. The number of graded pigs has increased since 1996 (*Figure 1*). The new method of carcass grading was introduced gradually in slaughter houses in 1996, thus showing a larger increase in the number of carcasses graded. There has been a slight seasonal effect with an increase in winter and early spring and a decrease in late spring. However, large changes were usually caused by an increased import of pig carcasses accompanied by substantial subsidies from the importing country. In such cases, a large decrease in the number of pigs slaughtered was followed by a substantial increase in a very short time. The most severe case happened in 1999. Since 1996, the production cycles were very irregular and unpredictable, causing many problems in pig industry.

Figure 1

Number of pigs slaughtered in Slovenia between 1996 and 2005



Changes in pig industry can be extracted from the structure of pig suppliers (*Table 7*). Pigs were sold to the meat industry from 278 suppliers in 2005. Changes have been observed graduately since 1996, however, much larger in the last period. Thus, the number of suppliers was reduced for one third from 2004 to 2005. The reason may be explained by large initiative of some large farms to organize pig production also in the small sector. Some farms have increased reproduction part and organized fattening on family farms, while the others have extended cooperation with smaller stockholders also to reproduction. Pig industry is concentrating around large units lately. Thus, only 7 suppliers (2.52%) sold more than one third of slaughtered pigs (38.50%) last year. The proportion increased for about 5% compared to 2004. Suppliers with less than 1000 pigs slaughtered per year were numerous (80.85%) but sold only 14.46% of pigs which had on the average less lean meat and were also more variable in quality.

Table 7

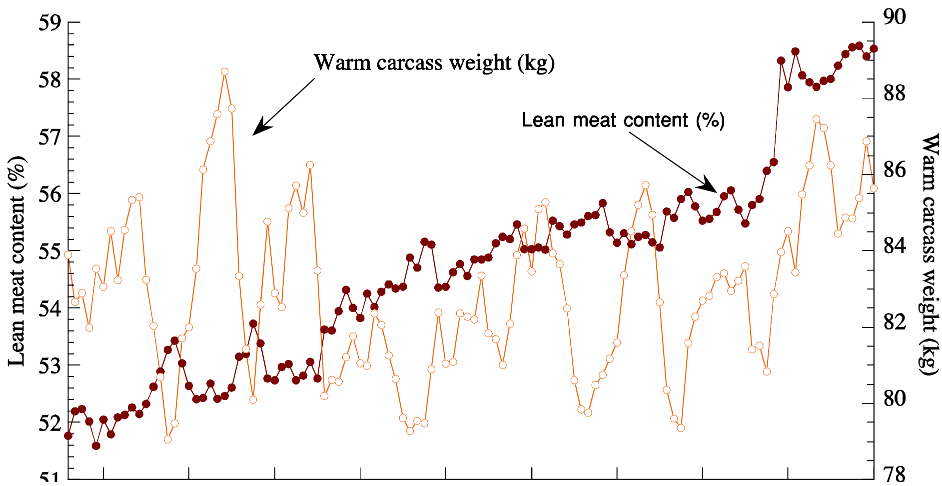
Distribution of suppliers by number of pigs slaughtered in 2004 and 2005

No. of pigs slaughtered per year	**2005**			**2004**		
	Suppliers (%)	Pigs (%)	Lean meat (%)	Suppliers (%)	Pigs (%)	Lean meat (%)
> 10000	2.52	38.50	58.15	1.52	32.97	56.16
7000-10000	2.16	15.21	58.97	1.53	13.91	56.64
4000-6999	2.52	11.31	58.24	1.52	8.41	56.79
2000-3999	5.76	12.36	58.60	4.83	16.04	56.18
1000-1999	6.47	8.17	58.44	6.85	11.05	56.59
500-999	11.15	6.98	57.96	10.15	8.37	55.90
100-499	30.22	6.35	57.35	28.17	7.51	55.76
50-99	11.87	0.70	56.83	11.68	0.99	55.70
< 50	27.34	0.43	56.02	33.76	0.75	55.00
Total	278	368690	58.28	394	379125	56.27

Changes in carcass quality can be described by two aspects (*Figure 2*). The first one is carcass weight which showed slow long term increase in the last years. The average carcass weight in 2005 was 85.8 kg and was increased about for 5 kg. The increase of overall variation can be justified by the tendency of some slaughter houses to get heavier pigs for ham. However, the producers slightly decrease a variation with groups. Large seasonal changes of carcass weight over years were observed. Seasonal changes in weight are closely related to the market situation. The second aspect of carcass quality was connected to lean meat content. As seen from *Figure 2*, lean meat content was constantly improving from around 52% in 1996 to over 58% in 2005. The larger step in 2004 was caused by renewed equation for DM5 and will be explained later. The changes were due to improvement of fattening conditions like reduced density, better housing conditions, splitting fatteners by sex and/or genotypes, and improving feeding regimes as well as diets. Some changes were also caused by changes of genotypes in the population. Latelly, we have recorded much smaller pure breed populations, increased use of Pietrain as terminal sire breed and increased terminal hybrids containing Pietrain breed as well. The production data collected regularly did not give a possibility to determine the importance of each factor.

Figure 2

Changes of warm carcass weight an lean meat content since 1996



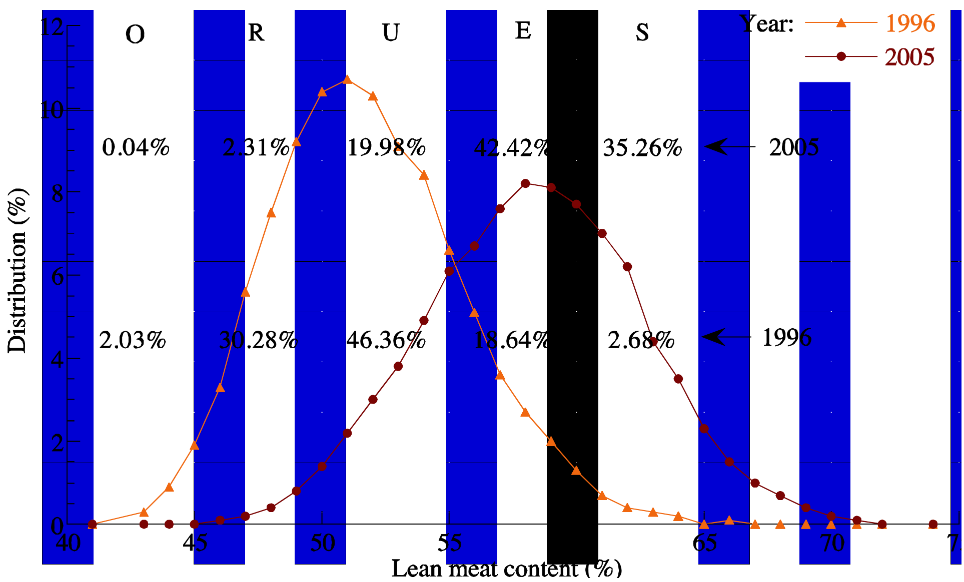
Lean meat content changed about 6% in 10 years causing large changes in distribution of carcasses over grading classes (*Figure 3*). In 1996, only 21.32% of carcasses were assigned into class E and S while ten years later the two upper classes contained altogether 77.68% carcasses. Almost the same proportion of carcasses (76.64%) was allocated into classes U and R in 1996. The standard deviation of lean meat content was increased form 3.83% to 4.26% and was mainly due to large variation in carcass weight.

Some major changes in carcass quality can be explained from trends in lean meat content (*Figure 4*), measurement M (*Figure 5*) and S (*Figure 6*) observed at different weights. Carcasses were observed on 5 kg interval for warm carcass weight between 70 and 95 kg. Independently of carcass weight, all three traits were improving over years.

Changes for predictor traits M and S were changing more or less constantly. Trait M was enlarging by rates between +0.41 mm/year at 70 kg and +0.60 mm/year at 95 kg and advanced more at heavier carcasses. The change may be caused by the improvement of genotypes or faster growth reducing some critical points in fattening. Trend for fat thickness (measurement S) was decreasing gradually up to 2002. Since than, trends were almost none for two years. The average trend was -0.57 mm/year at 70 kg and -0.74 mm/year at 95 kg. The changes were again more evident at heavier carcasses. The carcasses of 95 kg had more subcutaneous fat (7.5 mm) than carcasses of 70 kg in 1996. The difference was reduced to 5.5 mm in 2005. Changes in fat were caused to a large degree by environmental components influencing growth rate and body composition.

Figure 3

Comparison of lean meat content in 1996 and 2005



There was a sudden increase in lean meat content in 2004. It was mainly due to the adjustment of prediction equation used by DM5 grading method. The adjustment of formulae was needed because most carcasses were underestimated with the old equation. On average, the bias was 1.7%, however, almost half of the carcasses were assigned into lower grading classes. Thus the reduced trend since 2000 can be explained by increasing bias due to the deficiency of prediction equation.

In the future, we do not expect many changes in average lean meat content. It is intended to focus on the reduction of variability in carcass weight as well as leanness. Nevertheless, it is neccessary to define carcass characteristics required for special products like various hams and other traditional products. Pigs used for fresh meat, which are slaughtered at 100 kg live weight, are usually not appropriate for most of dried and smoked products. There are still large improvements possible in some production traits, like fertility, growth, and pig survival.

Figure 4

Trends in lean meat content over time at different carcass weights

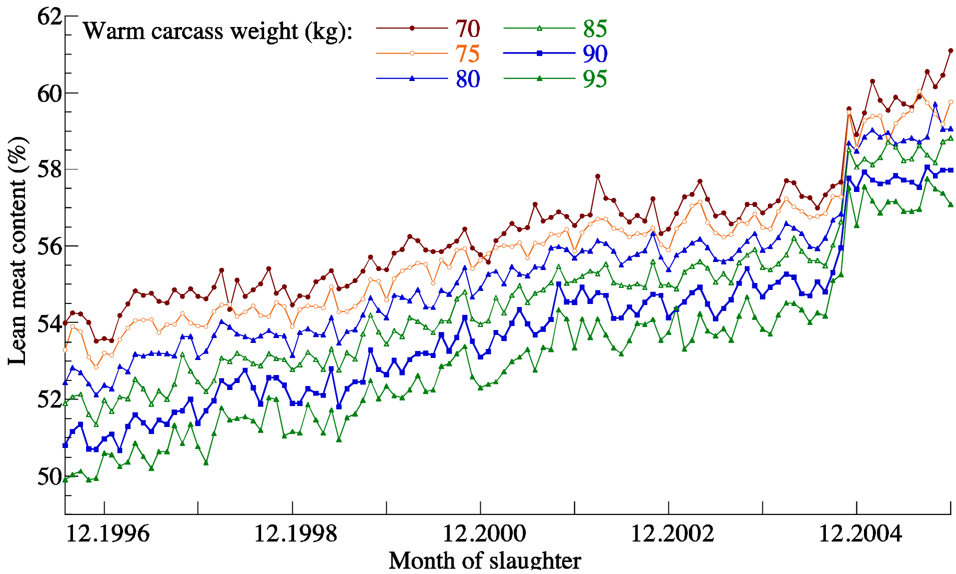


Figure 5

Trends in measurement M over time at different carcass weights

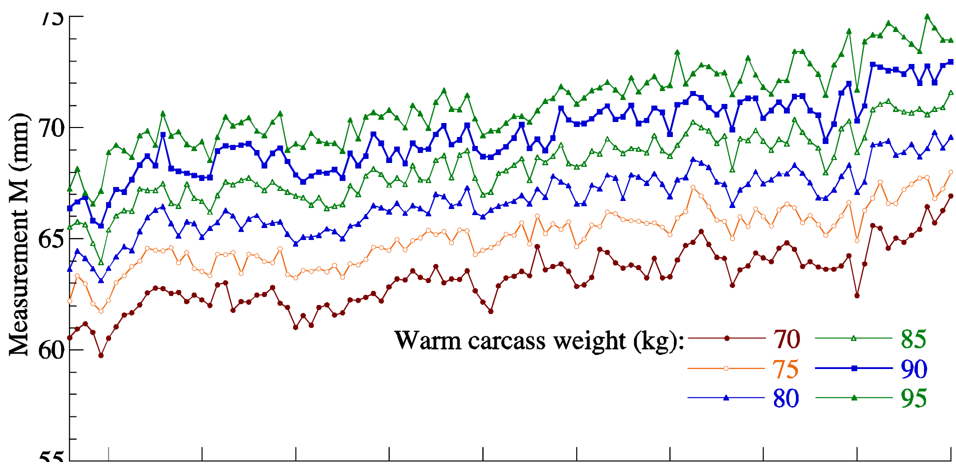
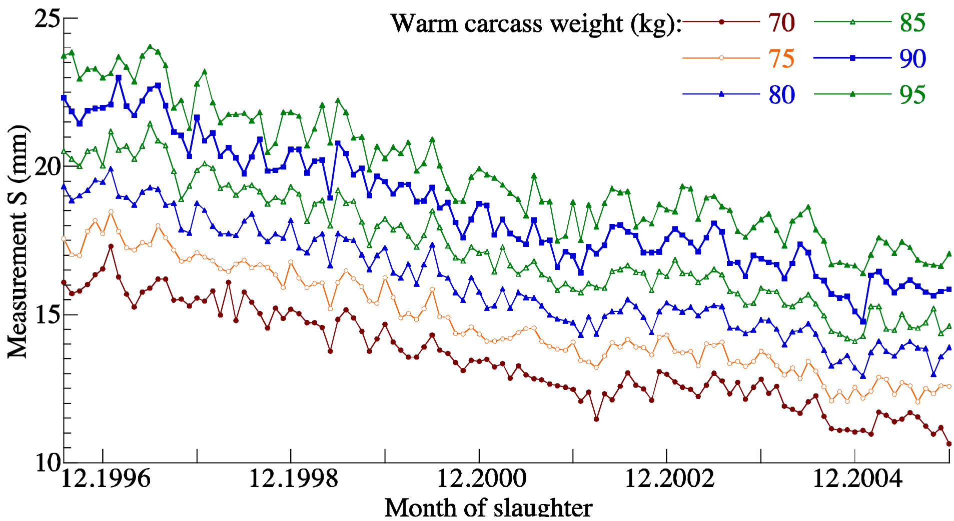


Figure 6

Trends in measurement S over time at different carcass weights



SHEEP AND GOAT MEAT PRODUCTION

Sheep and goat meat production is based on two types of production:

– Suckling lamb and kid production by milk breeds (Bovška breed and Istrian Pramenka sheep breed, and Slovene Saanen and Slovene Alpine goat breed). The average live weight by slaughter is less than 22 kg.
– Lamb and kid production by meat breeds (Jezersko-Solčava breed, Improved Jezersko-Solčava breed and Bela Krajina sheep breed ,and Goat Boer breed). The average live weight by slaughter is between 25–40 kg.
– In last years the most effective way is industrial crossbreeding with Texel and Charollais breeds.

POULTRY MEAT PRODUCTION

In Slovenia like in most of the world, the great part of poultry meat is chicken. In the last decade several changes in quality of poultry meat have been noticed in accordance with veterinary and health trends. Owing to its specific composition poultry meat is regarded as healthy and light food. Healthy and functional foods are now the main trends in poultry product trades. Better quality of poultry meat has been achieved by the following changes in *poultry nutrition*:

– no fish meal and other animal proteins are used,
– no antibiotics,
– some enzymes are added to achieve better absorption of minerals and nutritive matters from other cereals (wheat).

Several tests of probiotic and prebiotic additives that affect intestine microflora and digestion in animals and can, therefore, prevent some diseases in people have already been done; the mentioned additives are not used in nutrition at present; addition of minerals (organic selenium – better feed conversion) and vitamins (HyD – better bone firmness); additives are not used in production.

A constant quality of feed enables a constant quality of meat and fatness of chickens. The insertion of home produced feed in larger poultry companies prevents changes in quality of poultry meat production. A considerable advancement was noticed in *safety and constant quality of products:*

- certificates that ensure higher level of safety and quality of meat and products were achieved (HACCP, EFSIS, BRC…);
- strict veterinary and sanitary conditions in the whole chain of production that have already been introduced diminish health risks;
- permanent in-service training of employees in hygienic treatments of foods diminishes possibilities of later contaminations;
- uninterrupted cooling during poultry processing preserves constant quality and prevents microbiological risks;
- some technological changes like elimination of water-cooling system, higher quality of treatment and lower rate of damages contribute to better quality of meat;
- animal welfare,
- strict microbiological monitoring diminishes microbiological risks.

A *special product* is “maize chicken”, i.e. a typically yellow coloured chicken reached by feeding meals with at least 50 % of maize. Some markets require “aged fillet” that has very tender and soft structure attained by a specific way of production.

Most chickens are reared in intensive indoor production. The biggest poultry company in Slovenia started to produce *free range chickens* at their cooperative farmers in 2001. The chickens are certified as “better quality”. The free range system requires at least one m^2 of grass-covered area per bird in the second part of breeding but due to danger of aviary influenza in domestic poultry this system of breeding is not used at present. The results of our researches with free range chickens have shown that:

- free range chickens were significantly less fat than extensively indoor reared chickens (*Holcman et al*., 2003). Samples of breast with skin of the free range chickens contained significantly less fat and more minerals than samples from indoor-raised chickens. The instrument-measured cutting values were significantly higher in the free range chickens (*Rajar et al*., 1999).
- Free range broilers showed a higher degree of pigmentation in skin colour than the broilers in confinement. The differences were significant for the L* (lightness) and b* (yellowness) values (*Terčič et al*., 2000).
- From the point of view of human nutrition, free range broilers gave meat with better fatty acid composition (increased content of n-3 and n-6 fatty acids) compared to indoor raised chickens. Fatty acid composition was more favourable for breast than leg (*Polak et al*., 2002).

Some breeders have recently started to fatten chickens according to ecological standards, and others produce capons. In the past the breeding of capons of Slovene autochthonous breed of Styrian hen was very popular. The meat was famous for its tastiness and juiciness. Hence our aim is to renew the production of capon meat.

Due to the awareness of consumers and their health care the consumption of safe, healthy and functional products that satisfy their needs and do not cause further diseases will prevail. The present trends are functional poultry production and poultry meat products, which are followed by poultry companies in Slovenia as well.

MILK PRODUCTION AND QUALITY

According to the report by Slovene Dairy Association (GIZ Mlekarstva Slovenije) which combine 7 dairies, 383 million litres of milk were bought-in from 24.165 producers by Slovenian dairies in 1996, and 448.6 million litres of milk from 8.908 producers in 2005. Compared with previous five years, the bought-in quantity of milk was increasing progressively and reached the highest rate of buying-in in 2004 (486 million litres), and then decreased approximately to the level of 445.5 million litres in 2000 once more. The main reason for decrease of buying-in milk in 2005 was export of raw milk by the producers association. Slovenian dairies bought only 88.25% of delivered milk in the year 2005. *Table 8* shows the quantity of produced and delivered milk in Slovenia.

Table 8

Quantity of produced and delivered milk (1000 Litres) in Slovenia from 2000 to 2005 (*Statistical Office of the Republic of Slovenia*, 2006)

Year	Produced milk	Delivered milk
2000	629.736	453.896
2001	633.820	472.765
2002	706.446	487.683
2003	642.380	498.705
2004	631.456	503.348
2005	635.000	508.341

More strict regulations and new systems of payment by quality have improved the milk quality. In 1996, 78.8% of bought-in milk was classified into the category of European milk quality, i.e. the total number of micro-organisms in 1 ml of milk was lower than 100.000 and with 62.9%, the number of micro-organisms in 1 ml of milk was lower than 50.000. Only in 5% of bought-in milk, the number of somatic cells in 1 ml exceeded 600.000, and in 75% of milk the number of somatic cells in 1 ml was lower than 400.000 (*Slovene Dairy Association*, 2006). The goal was to achieve the European milk quality; that was the only way to be able to trade with other countries. The Council Directive (92/46/EEC) published on 16 June 1992 included all the aspects of quality assurance for milk and milk products, from milk production and placing of products on the market.

Rules on lying down the bacteriological quality of foodstuff on the market (Official Gazette of the RS, No. 39/92) was the first step in improving the quality of milk. They described that since 1994 the total bacterial count of raw milk should be 100.000 of m.o./ml not 3.000.000 m.o./ml allowed before.

In 1994, the country started paying the bacteriological milk quality dependent upon the level achieved by individual producers at the time of supplying milk into the dairy. The raw milk was classified into quality grade on the base of total bacterial count. The stimulation was different. The quality up to 50.000 m.o./ml was stimulated for 10%. The quality of milk from 50.000 m.o./ml to 100.000 m.o./ml was declared as normal quality and was not stimulated. In the same year, the determination of bacteriological milk quality by automatic epifluorescent microscopy by means of the instrument BactoScan 8000 was introduced in Slovenia. In 1995, we joined the network for comparability of the results of bacteria cell measurements, liaising 12 European countries (104 instruments) at that time and 18 laboratories (84 instruments) in 2006. The comparison was organized by Milk Standard Service (Hüfner), Germany, now Milchwirtschaftliches Institut Dr. Hüfner (MIH). The comparability of bacteriological milk quality measurements at the international level is confirmed in this way.

The raw milk quality has been improving due to the increased payment of milk by quality and by raising the criteria (Rules on laying down the cow milk purchase price, Official Gazette of the RS, No. 34/93, No. 72/93, No. 68/94, No. 16/96, No. 41/96). *Table 9* shows the share of milk in relation to bacteriological quality.

Table 9

Share of milk (%) in relation to bacteriological quality in Slovenia from 1994 to 2005 (*Slovene Dairy Association*, 2006)

Year	Share of milk, % to 50.000 m.o./ml	Share of milk, % to 100.000 m.o./ml
1995	60.99	78.10
1996	63.31	79.03
1997	68.61	83.05
1998	69.89	84.60
1999	70.02	85.97
2000	85.78	95.16
2001	90.55	96.74
2002	91.21	97.35
2003	91.81	98.12
2004	92.94	98.57
2005	93.72	98.52

Milk producers decided to adopt the so-called directed dairy farming. Smaller producers who could not meet the quality criteria, started to abandon the production. The year 2000 was a turning point.

In 1996, the average milk in Slovenia contained 3.98% of milk fat, 3.24% of proteins, 8.55% of non-fat solids and the average freezing point was -0.521 ºC (*Golc Teger*, 1998). In 1993, payment of protein content as part of the system for payment of milk by quality started (*Golc Teger*, 1998). The protein content in bought-in milk after 1995 increased to the average level of 3.36% in 2005 (*Slovene Dairy Association*, 2006). The fat content in bought-in milk reached the average level of 4.10% in the period following the year 1999, and reached the level of 4.15% in 2005 (*Slovene Dairy Association*, 2006). The quantity of proteins and fat content in bought-in milk in Slovenia are shown in *Table 10*.

Table 10

Buying-in milk (millions litres) and quantity of proteins and fat content in milk in Slovenia from 1999 to 2005 (*Slovene Dairy Association*, 2006)

Year	Buying-in milk	Proteins %	Fat %
1999	434.9	3.35	4.10
2000	445.5	3.36	4.10
2001	459.0	3.34	4.12
2002	473.5	3.33	4.13
2003	484.2	3.34	4.14
2004	486.0	3.36	4.16
2005	448.6	3.36	4.15

The rules on the milk quality in force state the freezing point of -0.520 ºC as the basic criterion to assess the raw milk quality (Official Gazette of the RS, No. 21/93). The criterion in 1994 was the value of freezing point lower than -0.515 ºC (*Golc Teger*, 1998).

The number of somatic cells as a criterion for milk quality and a parameter for payment of bought-in milk was introduced in 1994. The share of milk at the time of buying-in with the number of somatic cells in ml of milk lower than 400,000 was constantly increasing after 1996 and amounted to 91.2% in 2000; in 1.1% of milk, the number of somatic cells in ml was lower than 600.000. In 2005, 91.8% of milk reached the level of less than 400.000 somatic cells in ml and only 1% of milk reached over 600.000/ml. *Table 11* shows the share of milk in relation to somatic cells count.

Table 11

Share of milk in relation to somatic cells count in Slovenia in the period from 1997 to 2005 (*Slovene Dairy Association*, 2006)

Year	Share of milk, % to 400.000 sc/ml	Share of milk, % over 600.000 sc/ml
1997	83.37	3.48
1998	82.06	3.06
1999	85.15	2.16
2000	91.15	1.07
2001	93.39	0.81
2002	92.76	0.88
2003	92.19	0.82
2004	92.72	0.80
2005	91.81	0.93

Slovenian laboratories are checking the quality of analyses and of micro-biological tests performed by integration into the international inter-laboratory comparative tests, which have been organised by recognised institutions and associations (MUVA, Kempten, CECALAIT, Poligny, AIA, Rome, AFEMA, Vienna) since 1992.

In the future we expect to maintain the quality control of milk for payment at the time of buying-in and for the selection service performed in central laboratories. Development of analytical methods will be directed towards supporting dairy farm management by selecting additional parameters, important to achieve the required quality (for example: determination of urea, free fatty acids, etc).

CONCLUSIONS

- Meat and milk production has stabilised in the last years.
- The quality of slaughtered cattle, estimated on the basis of noted conformation and fatness, has worsened
- On the contrary, very big improvement in quality of slaughtered pigs was noted. Meat percentage increased for almost 5%.
- Sheep and goat meat production increasing about five times in the last 15 years
- Poultry meat producers have focused their efforts to satisfy the consumers needs for safe, healthy and functional products.
- Te quality of milk regarding the number of total bacterial and somatic cell count greatly improved.

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Compound feed as a factor influencing the food quality and safety

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ABSTRACT

The paper discusses on the basis of data from the literature and the results of the author's own research work how animal nutrition can be used in the "from farm to fork" chain to influence the quality of pork and poultry meat and the safety of animal products. The relevant results show, that for example in pig nutrition different ileal digestible lysine/energy (IDLYS/DE) ratios will essentially determine the fat content of meat and consequently the quality of the product. The article also highlights the fact, that the dietary IDLYS/DE ratio of genetically improved pigs substantially differs from that of the normal hybrids. While in the diet of a genetically improved pig the required IDLYS/MJ DE ratio is 0.7 g during the first phase of fattening (20–55 kg) and 0.6 g in the second phase of fattening (55–100 kg), these ratios for the normal hybrid are 0.6 and 0.5 g IDLYS/MJ DE, respectively for the two weight categories. This paper also warns, that if animal feed proteins, animal fats and various feed additives are used in a unprofessional manner, they may present a potential risk factor for the safety of the animal food product. The results of the studies discussed lead to the conclusion, that in the "from farm to fork" chain it is extremely important for the safety of products to define clear and unequivocal quality criteria and to establish an official and comprehensive control for each element of the product chain. The author proposes to conduct further systematic studies in accordance with the "from farm to fork" concept in the interest of producing high quality and safe animal food products.
(Keywords: animal nutrition, feed quality, food quality, food safety)

INTRODUCTION

In recent years almost everyone in the world is affected by various nutrition related health problems even if due to very different causes. In developed societies overeating and/or an imbalance of nutrients in the diet have led to the spreading of chronic, non-infectious diseases. In less developed societies under-nutrition causes major health problems. According to Hungarian statistics about 50% of mortalities are due to cardiovascular diseases and 30% to tumor diseases, in the pathology of which nutrition is one of the most important risk factors.

The existing research data already witness that the quality of foods of animal origin is influenced to a large extent by the quality of feed. While the world's compound feed output for 1975 was 290 million metric tons, this increased to 626 million metric tons by 2005 (*Gill*, 2006). This change is indeed striking despite the fact that the growth of production volume has slowed down in recent years.

A major portion of animal food products for the Earth's population is produced with this compound feed. The volume and quality of food produced using compound feeds is therefor by no means negligible. The purpose of this paper is to discuss how the composition, nutrient content of compound feeds, the balance of dietary nutrients influences the quality and safety of animal food products. Due to its magnitude the issue is presented primarily through pork production as an example.

MEAT QUALITY AND FOOD SAFETY

A study of the relevant literature shows that there is no uniform definition of meat quality. It is defined in general as the aggregate of the sensory, nutritional, toxicological, hygienic and technological characteristics of the meat. According to *Hofmann* (1993), however, meat quality comprises those characteristics and parameters of meat which are important from the aspect of nutritional value, human health and processing technology.

It is also obvious from the literature that meat quality is a different concept for the different participants of the production and retail chain. On this basis we differentiate hygiene, technology, sensory and nutritional value related meat quality (*Babinszky*, 1996). When examining the nutrition-physiological aspect of meat quality the definition will be different although not fundamentally. Nutrition physiology prefers low-fat meats, but another important criterion is that the meat intended for the market should be free of toxins, feed hormone preparations and other substances deleterious for the human body. This definition already involves the issue of food safety as well. Food safety is essentially the concept that the food should not be harmful for its consumer. The harms are usually classified into three groups (*Biró*, 2000):

1. *Harm caused by live agents*: this is a factor subject to the microbiological status of the food, when food bacteria may cause food poisoning, fungi may cause mycotoxicoses, viruses and parasites may damage health.
2. *Harm caused by chemical substances, toxins*: chemical contaminants (residues) can be herbicides, veterinary pharmaceuticals, chemical pollutants, excipients, etc.
3. *Harm caused by other contaminants*: in the course of processing the food may be contaminated with metal or glass fragments.

In summary it can be concluded that food safety is influenced by biological, chemical and physical factors (*Biró*, 2000). It should be noted however, that there is general agreement in the literature that the acceptability of a given food product incorporates the criteria of safety and of quality. In other words: even though safety and quality are separate units in their characteristics, at the same time there are numerous linkages between the two sets of criteria (*Biró*, 2000). This means that the two ideas are inseparable which should be borne in mind when producing foods of animal origin in animal agriculture.

THE RELATIONSHIP BETWEEN ANIMAL FEEDING, FOOD QUALITY AND FOOD SAFETY

In the last 10 years animal nutritionists have frequently dealt with the question of how animal nutrition can be used for influencing the quality of pork for example so that it may best satisfy the criteria of human nutrition. The experiences show, that the quality of animal food products, and thus of meat can be influenced, i.e. improved or deteriorated by feeding - just as by other environmental factors. Of the various nutritional factors affecting meat quality the method and intensity of feeding, the quantity and quality of nutrients fed and their relative proportions should be highlighted. It should also be noted, that the various chemical additives and pharmaceuticals used in a non-professional manner may also exert a negative influence on the quality of meat. The effect of animal feed on meat quality is presented through the following examples.

Polyunsaturated fatty acids

Omega-3 (ω-3) polyunsaturated fatty acids (PUFA) have become a focal point for research studies conducted in the fields of animal nutrition and human nutrition, both.

The results of clinical studies have shown, that consumption of these fatty acids will alleviate or completely eliminate certain diseases (coronary heart problems, psoriasis, certain inflammatory conditions). It also improves the development of sight in healthy and prematurely born infants, the dermal health status of adults, brain functioning and the productions of certain hormones (*Barlow* and *Pike*, 1991).

Fish oils contain ω-3 polyunsaturated fatty acids in abundance. The results of numerous studies have shown that when the diets of growing/finishing pigs or of broilers are supplemented with fish meal or fish oil, the level of long-chain ω-3 polyunsaturated fatty acids in the meat will increase in response (*Hulan et al*., 1988, 1989; *Chanmugam et al*., 1992; *Arbuckle et al*., 1994).

A potential problem is, that diets containing up to one percent of fish oil already may lead to a non-desirable off taste. In several countries the traditional consumer preference is for pork as opposed to fish, and consequently the consumers are more sensitive to any "fish taste" in pork. Linolenic acid is a short-chain ω-3 PUFA to be found in large quantities in vegetable oils, for example in rapeseed oil. The "double-zero" (low erucic acid and low glucosinolate level) and low fiber varieties of rape can be successfully fed to pigs and broilers. The research results suggest that rapeseed oil incorporated in the diet at 6% may increase the ω-3 fatty acid level of pork and poultry meat.

The influence of animal feed on the protein and fat content of meat

From the aspect of human nutrition physiology the protein and fat level, their ratio to each other, and the level of intramuscular fat are probably among the most important parameters. The protein and fat deposition of growing/finishing pigs is influenced by several factors. The most important of these is the genetic potential of the animal for protein deposition and for feed intake. The relationship between these two factors is discussed by several theories. Of these a commonly used concept is the so-called linear-plateau theory published by *Bikker* (1994) and shown in *Figure 1*, according to which protein deposition increases linearly with the energy intake up to the limit of the genetic potential for protein deposition.

The growth performance and the chemical composition of the carcass (the meat quality) is also affected by the amino acid/energy ratio of the diet. It is well known, that for pigs the primary limiting amino acid is usually lysine. *Table 1* illustrates the strong correlation between ileal digestible lysine intake and average daily weight gain, and also between the daily protein deposition and the feed conversion rate (*Halas* and *Babinszky*, 2000). In consequence it is indispensable that we aim for creating the best possible lysine / energy (DE) ratio during diet formulation in order to enhance protein deposition. The trial results show, that in the case of growing pigs (between 25 and 60 kg of live weight) the lowest fat deposition level can be expected with an 0.63 g ileal digestible lysine/MJ DE ratio. The data from these studies suggest, that any deviation from this lysine / energy ratio will lead to a higher fat content of the carcass and consequently to the deterioration of the meat quality. The results of the relevant studies also show, that the ratio determined for the 25–60 kg live weight will decrease to 0.50 g ileal digestible lysine/MJ DE during the second phase of fattening (between 60 and 105 kg of live weight).

Figure 1

Linear-plateau and curvilinear relationship between protein intake and protein deposition in case of two different energy intakes (*Bikker*, 1994)

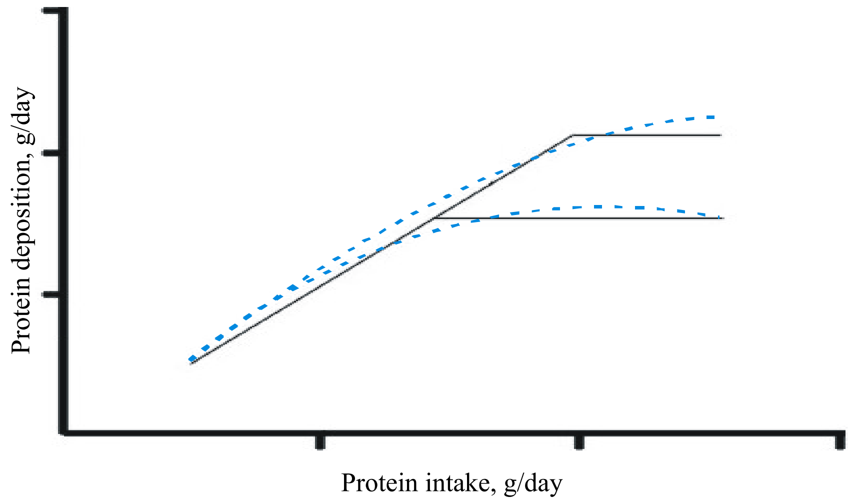


Table 1

The relationship between daily ileal digestible lysine intake, daily weight gain, daily protein deposition and feed conversion ratio (FCR), (*Halas* and *Babinszky*, 2000)

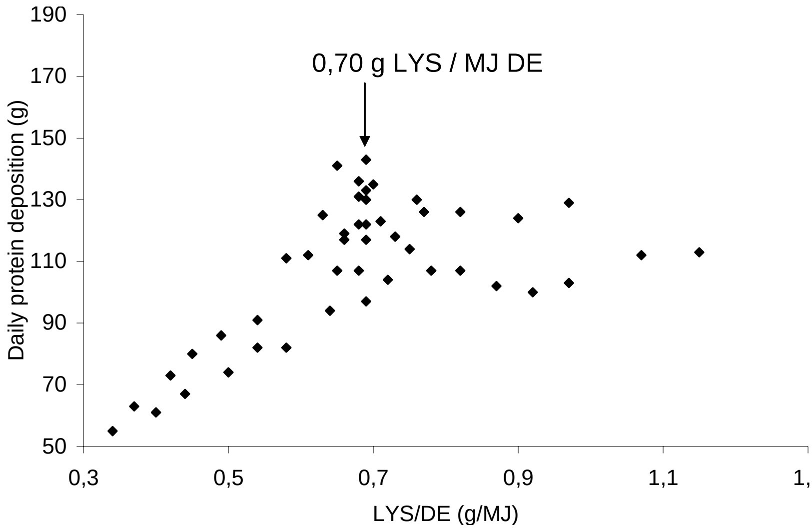
Body weight	Correlation		
	Daily weight gain	Daily protein deposition	FCR
30–60 kg	r= 0.94 P=0.0001	r=0.78 P=0.001	r=-0.94 P=0.0001
60–105 kg	r=0.89 P=0.0001	r=0.77 P=0.0013	r=-0.87 P=0.0001

The data in *Figure 2* are a summary of the relationship between total lysine/energy and protein deposition on the basis of various data from the literature (*Szabó*, 2001). Despite their rather high variance the data show that between 20 and 55 kg live weight the daily protein deposition is usually the highest beside an 0.70 g total lysine/MJ DE ratio, which corresponds to an 0.6 g ileal digestible lysine / MJ DE ratio. It should be noted however, that the foregoing lysine/energy ratios pertain to hybrids with a so-called average genetic potential (normal pig). Three categories were set up for hybrids in the literature (*Close*, 1994):

– Superior, genetically improved pigs;
– Normal pigs;
– Traditional, unimproved pigs.

Figure 2

The influence of dietary total lysine and digestible energy ratio on the protein deposition of growing pigs based on the review of literature (Szabó, 2001)



The average daily weight gains and protein content of the empty body characteristic of each category are shown in *Table 2*.

Table 2

Three categories of pigs have been identified depending upon their rate and composition of body gain (*Close,* 1994)

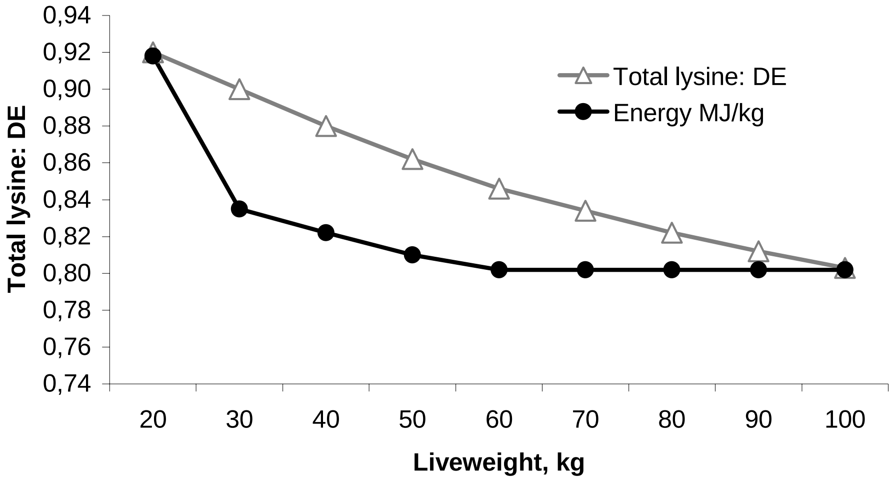
Categories	Growth rate (kg/d)	Protein content of empty body (g/kg)
Superior, genetically improved pigs	up to 1.2	180
Normal pigs	up to 1.0	170
Traditional, unimproved pigs	up to 0.8	160

Remarks: For animals it has been assumed that maximum growth rate is achieved at a body weight of 60 kg and is maintained constant thereafter up to 100 kg body weight that is, in a linear-plateau fashion.

Results of the studies conducted so far show, that when the lysine/energy ratio in the diet of hybrids belonging to the first category (improved pigs) is the same as in the feed of normal pigs, these will deposit access fat by the end of the fattening period, i.e. the quality of the meat will deteriorate substantially. For this reason *Varley* (2001) suggests to feed these pigs with a diet containing 0.7 g ileal digestible lysine per MJ DE during the first phase of fattening (between 20 and 55 kg of live weight), and 0.6 g during the second phase (between 55 and 100 kg live weight) (*Figure 3*).

Figure 3

Lysine and digestible energy requirements for improved pigs (*Varley,* 2001)



These data stress the importance of knowing the genetic potential of our growing/finishing herd, because this knowledge is indispensable during diet formulation for establishing a proper lysine/energy ratio so that the quality of meat can satisfy the criteria of human nutrition even in the case of the improved, high-producing pigs.

This perception has also contributed to the necessity of nutritionists, geneticists, molecular biologists, human nutrition biologists, physicians and biochemists all working together in order to produce quality meat. This research cooperation has yielded molecular nutrition as a new field in animal nutrition.

Some feed ingredients and feed additives as potential risks for food safety and potential solutions

The production of safe food (e.g. meat) also demands investigating whether the key feed ingredients and additives present any risk. With respect to the principal feed ingredients it is known, that grains may primarily be a risk factor due to toxins produced by various fungi. The number of already known mycotoxins exceeds one thousand, but the discovery of further mycotoxins is more than likely. Of the known ones approximately 100 mycotoxins have proven harmful effects, and of these about 15–20 are of outstanding significance in human and veterinary health. From the aspect of their harmful effects aflatoxins, fusarium toxins and mycotoxins produced by certain storage moulds are of major importance. Mycotoxins may be produced on the field prior to harvesting, and in case of suboptimal storage conditions also after harvesting. The prolonged feeding of contaminated grains may lead to an accumulation of toxins in the animal body. But even low levels of toxins may intensify their damaging effect through interaction impairing thereby the quality and nutritional value of the animal products, e.g. milk, egg, organs (liver, kidney, brain, heart, etc.) and to a lesser extent of meat as well.

No definitive protection against the harmful effects of mycotoxins exists as yet, but they can be alleviated by means of e.g. selective breeding, professional agro-technology, plant protection and storage practices. The attempts to bind the feed toxins inside the gastrointestinal tract with various additives in many cases are promising, but further systematic studies are required.

Dietary proteins of animal origin may mean a risk factor for the health of livestock and for producing safe animal food products. A case in point is the BSE scandal erupting a couple of years ago. The results of the relevant studies show however, that by complying with the animal health regulations and the technology standards of meat meal production, and with a strict control of this compliance this risk factor can almost entirely be eliminated. Another solution for supplying safe protein in adequate quantity and quality to the pig and poultry industries is to use more of the vegetable protein sources and to replace the animal protein sources with plant protein sources.
In pig nutrition for instance the proper way for replacing the animal protein sources with vegetable protein sources in the concentrates is if during diet formulation we first determine the energy content of the diet in accordance with the age and class of the animal, and then the ileal digestible lysine/DE ratio. Next we determine the ratio of the other amino acids to lysine using the ideal protein concept. The necessary amino acid percentages can be provided by incorporating industrial (crystallized) amino acids in the compound feed.

Another component to watch for in the diet formulation is that the digestible phosphorous content of plant protein sources remains below that of feed ingredients of animal origin. This necessitates that we use the digestible phosphorous value when formulating the diet and in order to enhance the digestibility of the plant derived dietary phosphorous it is advisable to incorporate a phytase enzyme at the level of 500 U/kg of diet. Provided that the diet of growing / finishing pigs is formulated on the foregoing basis, we need not expect any reduction in meat production or deterioration of meat quality when replacing protein sources of animal origin to plant protein sources (*Babinszky* and *Vincze* 2004).

Animal fats of uncertain origin and prepared with unsuitable technologies should also be treated as a risk factor both in poultry nutrition and in pig nutrition. A case in point is the dioxin scandal several years ago. The single most important dioxin contaminant source for poultry and pig is the feed ingredients of animal origin, and as first among these animal fat. According to the literature the dioxin content of animal fat is accumulated in the fat tissues of the animal by close to 90% efficiency. This chlorine containing aromatic chemical compound is an extremely aggressive toxic compound.
The solution for alleviating the harmful effects of dioxin is a careful selection of the fat source, compliance with the manufacturing technology standards and the relevant animal health regulations, together with a strict control of this practice. Replacing animal fat as an energy source with carbohydrates is also a viable solution, but the energy source should always be decided after a careful consideration by the nutritionist. This is necessary, because the relevant respiratory studies show, that when pigs produce body fat or milk fat from dietary carbohydrate (e.g. starch) the energetic efficiency is lower when they use dietary fat for the purpose. This is the reason why we experience a drop of feed intake in hot, poorly ventilated houses when high levels of carbohydrates are fed. In consequence of the lowered nutrient supply also the meat quality may suffer (*Babinszky*, 1998).

A wide variety of **additives** (e.g. antioxidants, flavour and colour enhancers, technological additives, various types of growth promoters, pharmaceuticals, and previously growth promoting antibiotics, etc.) are incorporated in today's compound feeds. Several of these can be considered a risk factor, such as for instance the antibiotics used as growth promoters.

It is well-known, that antibiotics are essentially banned in the EU for growth promoting purposes, because they may result in residues and resistance in the animal body, and may cause cross-resistance in the human body.

With the banning of growth promoting antibiotics their alternatives are increasingly in the focus of attention. **The alternatives of growth promoting antibiotics** can be listed under several groups, such as probiotics, prebiotics (oligosaccharides), or the mixture of the two, the symbiotics. Various organic acids, feed enzymes and herbal extracts (herbs, spices, essential oils) can also be considered as alternatives.

Table 3 presents the influence of feeding a probiotic product on the digestibility of nutrients and on nitrogen retention, on the basis of studies conducted with weaned piglets (*Babinszky* and *Tossenberger*, 2003).

Table 3

The influence of probiotics on the digestibility of selected nutrients and on N-retention in weaned piglets (*Babinszky* and *Tossenberger*, 2003)

Item	Treatment	
	Control group	Probiotic group
Digestibility (%)		
- crude protein	78.7^{a}	83.2^{b}
- crude fat	53.0^{a}	60.4^{b}
- N-free extract	91.6^{a}	91.9^{a}
N-retention (g/day)	12.9^{a}	14.5^{b}

^{a,b}Within a row, means without a common superscript letter differ (P<0.05).

Organic acids as an alternative of growth promoting antibiotics are frequently proposed in the feeding of weaned pigs. *Table 4* summarizes the results of studies conducted by *Babinszky et al*. (1998). During the trial 6.5 g formic acid/kg of feed and 9.8 g formic acid/kg of feed was incorporated in the diets of weaned piglets. The results of the tests prove that even in the case of 6.5 g formic acid/kg of feed the piglet performance improves significantly compared to the control (non-supplemented) group.

Table 4

The influence of formic acid on the performance of weaned piglets (*Babinszky et al.*, 1998)

Parameters	Treatments		
	Control	I*	II**
Average daily feed intake, g	591^{a}	616^{ab}	636^{b}
Average daily weight gain, g	303^{a}	341^{b}	358^{b}
Feed conversion ratio, kg/kg	1.98^{a}	1.83^{b}	1.81^{b}

*I. 6.5 g formic acid/kg diet; **II. 9.8 g formic acid/kg diet; ^{a,b}Within a row, means without a common superscript letter differ (P<0.05).

In general it can be stated therefore, that these preparations can be applied with an efficiency quite similar to that of the antibiotics, but it should also be noted, that the effect of these preparations is also depending to a large extent on the herd breed, age, class, health status and the type of diet fed. With herbal preparations a further difficulty can be that the active substance can not be identified exactly as a chemical compound, i.e. it can not measured precisely. For this reason in a part of the relevant studies not even the composition and dosage of the active substance is known. In consequence further extensive studies are required both in pig and in poultry (broiler) nutrition, and in the field of chemical analyses as well.

The "from farm to fork" food production chain in research work and in the production of animal food products

The feed examples discussed in the foregoing are suitable for solving one or another partial problem. In the interest of producing high quality and safe animal food products (e.g. meat) however, it is necessary nowadays to examine the entire chain both in research and in the production of animal products. Therefore the production of high quality and safe animal food products demands that already at the first link of the animal product production chain, i.e. at field crop production high quality and safe production practices are in place. Accurate information are required about soil management, plant protection, and whether GMO grain is produced on the farm in question. The next link in the production chain is the animal feed industry. At this step in addition to the feed ingredients of plant origin also the industrially manufactured feed ingredients and feed supplements need to be controlled. Furthermore, each step of the compound feed manufacturing process and eventual manipulations in the feed mill (such as hydrothermic treatment, extruding, expanding, micronizing or other) should be controlled.

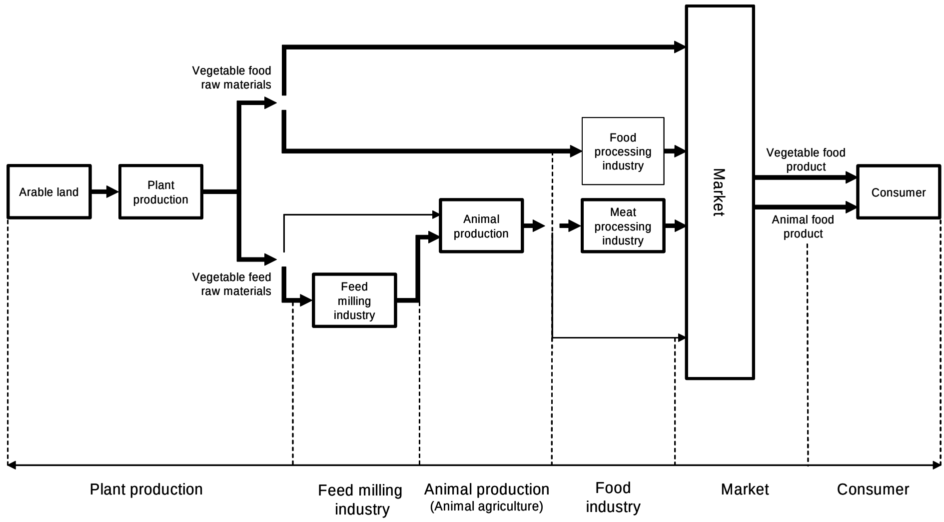
The resulting compound feed is transferred to the pig operation, where all important data of each phase in the feeding and fattening process must be recorded together with the herd data. Having reached the slaughter weight the herd is transported to the slaughterhouse or the processing plant. Here again each processing stage is controlled and the data are entered in the central terminal (data file) of the product chain, where upon the evaluation of the data it can be discovered immediately if the activities at a certain point of the production chain deviate from the regulations, or if the data measured do not meet the regulations and the quality criteria.

At the end of the product chain the output is a "food product of planned quality and safety derived from a planned feed" controlled at every stage of production, and which when delivered to the supermarket shelves and cold counters can also be verified by the consumers themselves with the help of a bar-code.

Figure 4 presents the entire food production chain outlined in the above under the title "from farm to fork". The purpose of this research and development and innovation (R+DI) project is to supply to the consumers animal products of the highest possible quality and safety. To this end however, crop production, feed industry, livestock production and the food processing industry and trade need to work in very close cooperation. Above all this it is also necessary that the researchers involved in the fields of animal nutrition, human nutrition, nutrition biology, nutrition immunology, molecular nutrition and also the information technology specialists work together.

Figure 4

The „from farm to fork" food production chain



Such a highly qualified research team committed by the foregoing philosophy will naturally be able to perform any high standard work only in case it possesses a high standard research basis, laboratories and a sufficiently comprehensive and accurate technical data base, quality criteria pertaining to each member of the production chain, and a high standard informatics background (software, hardware). This type of high level cooperation enables the controlling of every single point of the product chain in the interest of producing safe animal products.

In recent years the number of programs called "from farm to fork chain", "from field to consumer chain" or "from feed to food chain" is continuously increasing in the EU, the US and Canada, equally. It is a task of the near future that Central Europe should participate in these and similar research programs more intensively.

CONCLUSIONS

The following main conclusions can be drawn on the basis of the preceding chapters:

- high quality and safe animal food products can only be produced in case the elements of the product chain are built on each other in a planned manner and all participants of the production chain use the most advanced knowledge of their relevant field;
- it is a further precondition, that quality criteria are clearly defined, so that all elements of the product chain can be the subject of official and full-scope control. One of the most important criteria of producing high quality and safe animal food products is that on the basis of the "from farm to fork" philosophy we start dealing with the issue of food quality already at the field crop production stage;
- feed ingredients and feed additives may present a risk in the production chain, but these risk factors can be largely reduced through using expert knowledge and target-oriented control;

- also following from the "from farm to fork" philosophy it should be made clear already at the stage of feed ingredient production and compound feed manufacturing that the compound feed is intended for the production of animal food products. The quality of the animal product (human food) can be improved by the feed; but it can also be deteriorated by it;
- one "tool" for improving meat quality is target-oriented animal nutrition, but this is not a sole and exclusive precondition for achieving it;
- further systematic studies suiting the "from farm to fork" principle are required for producing high quality and safe feeds and animal food products.

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SECTION 1

PIG BREEDING

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Comparison of different methods for lean percentage evaluation in pig carcasses

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ABSTRACT

Present study was carried out on 64 pig carcasses selected according to backfat measures by instrumental method approved in Croatia. One day after slaughter the carcasses were dissected according to EU referent method. Dissected lean percentage was calculated by formula from Commission Regulation (EC) No 3127/94 and also by equations for estimation of lean percentage approved by Croatian Regulation. Lean meat percentage objectively determined by EU referent dissection method was 51.9% in average. When instrumental method and "two points" method were applied, estimated lean meat percentage was 55.1% and 56.6%, respectively. Comparison of dissection, as referent and objective method, with instrumental and "two points" methods for estimation of lean meat percentage showed that both estimation methods differ statistically (p<0.01) from dissection. The differences indicate overestimation of meatiness in the pig carcasses. There is need for establishment of new formulae for lean meat percentage assessment in Croatia.
(Keywords: pig, carcass, lean percentage, assessment methods)

INTRODUCTION

The pork market of EU countries has been based on meat percentage for a long time. Pig carcasses with higher lean percentage achieve a better market price. For estimation of carcass lean percentage at the slaughter line, different procedures are used. In these procedures, on the basis of certain measures taken from pig carcass, the share of lean meat is calculated according to official verified mathematical equation.

In order to obtain the formula by which the meat percentage of pig carcasses can be estimated with highest reliability, it is necessary to carry out comprehensive dissection experiment. The criteria regarding the size of sample and the accuracy of estimation are regulated by EU legislative (Commission Regulation No 2967/85).

In Croatia, the estimation of pig carcass quality and payment on the basis of so called meat unit has been carried out since 1973. The first Regulation on pig carcass quality which applies methods for pig carcass classification into market classes (SEUROP) at the slaughter line according to lean meat percentage (%), compatible to EU model has been adopted in 1995.

Two methods (instrumental and "two points") and respective mathematical formulae for meat percentage estimation of pig carcasses were given by this Regulation. Equations approved by the Regulation were taken from German regulations, but both of them were previously submitted to evaluation of accuracy in carcass lean percentage estimation when applied to Croatian pig population (*Petričević et al.*, 1993). *Kralik et* *al*., 1997 and *Kusec et al*., 1998 published original formulae for "two points" method, constructed from dissectional data obtained on Croatian pig populations.

Until recently, only "two points" method by which muscle and fat thickness are measured manually with steel tape was used in Croatian abattoirs. Although nowadays automatic devices for measuring muscle and fat thickness are used, statistically verified equation, obtained by dissection of regular number of pig carcasses, has still not been established. At the same time, there is no legal obligation in Croatia by which the payment of pig carcasses should be based upon the determined market class i.e. meat percentage.

Equation coefficients are dependent on population lean meat mean, and after two years time period or after significant alteration in pig population they should be checked (Commission Regulation No 2967/85). *Čandek-Potokar et al*. (2004) reported an important increase of average lean meat percentage in the carcasses of Slovenian pig population. Consequently, the percentage of pig carcasses being graded into S and E classes was almost tripled from 1996 to 2004. Authors argued that the improvement was initiated by the payment according to carcass lean meat content and that further improvements can be expected when Hennessy optic probe is introduced. For the purpose of verifying or establishing a new estimation formula, new European regulations introduced simplified EU dissection method for objective establishment of lean percentage in pig carcasses (Commission Regulation No 3127/94, *Walstra* and *Merkus*, 1995). The change of dissection method and a positive shift in carcass leanness stimulated the renewing of methods for assessment of lean meat percentage in carcasses of Slovenian pigs; the equation was announced to get in use during 2004.

Since the formulae for lean percentage estimation in Croatia have not been corrected for a long time and population mean of muscle tissue share in pigs is probably altered, the aim of this paper is to compare the results of estimated lean percentage by two methods in use with the new referent dissection method prescribed in EU countries.

MATERIALS AND METHODS

The experiment included 64 pig carcasses originating from gilts and barrows produced at Croatian large industrial farms and small family farms. The carcasses were selected according to back fat measures by instrumental method approved in Croatia (Regulation No 119/1999) at different Croatian slaughterhouses. There was no stratification according to carcass weight. Measures for the estimation of lean meat percentage by instrumental and "two points" (TP) method were taken at the slaughter line. For the instrumental method of lean percentage estimation, following measures (n=60) were taken:

- F: the thickness of backfat (including rind) in millimetres, measured at 7 cm off the midline of the split carcass, between the second and third last ribs,
- M: the thickness of the muscle in millimetres, measured at the same time and in the same place as F.
- Measures for TP leanness prediction (n=62) were:
- F: the minimum thickness of subcutaneous fat (with skin) at the split of the carcass in millimeters, above *M. glutaeus medius*,
- M: the thickness of lumbar muscle at the split of the carcass in millimeters, measured as the shortest connection between the cranial end of the lumbar muscle and dorsal edge of the vertebral canal.

Left sides of the carcasses were dissected according to EU referent method one day after slaughter (n=64). Four main parts (ham, shoulder, loin and ribs) were dissected into muscles, bones, intramuscular fat and subcutaneous fat with skin; tender loin is taken into calculation as separate part. Share of meat was calculated by formula from Commission Regulation (EC) No 3127/94.

Referent lean meat percentage = 1.3×100×weight of tender loin + weight of lean (fascia included) in shoulder, loin, ham and belly/weight of tender loin + weight of dissected cuts + weight of remaining cuts.

Lean meat percentage was also calculated by equations that are approved by Croatian Regulation (N.N. No 119/1999). These equations are referred to estimation of lean meat percentage by instrumental and "two points" methods. The obtained data were statistically processed by GLM procedures of SAS program package, version 9.0 (*SAS* Ins. Inc., 2002).

RESULTS AND DISCUSSION

The data collected in dissection experiment were statistically processed and the results are presented in *Table 1*. The weight of the warm carcasses was between 53 and 109 kg, with average value of 80.6 kg. This indicates large variation in slaughter weights of Croatian pigs and a need to act in the direction of increase in uniformity. The carcass weight is not included as an adjustment factor in payment system, which should be reconsidered if higher uniformity of carcasses is desired. The mean backfat thickness in experimental sample was 17.5 mm. The thickness of *m. longissimus dorsi* was measured in the same way; the mean value in investigated sample of pig carcasses was 53.9 mm. With these measures and by application of formula prescribed by Croatian national Regulation, the lean meat percentage of investigated pig carcasses was calculated; mean of the estimated lean share was 55.1%. Backfat thickness (14.7 mm) and the muscle thickness (68.4 mm) taken for estimation of lean meat percentage by "two points" method were also measured. Mean estimated lean percentage obtained by this method was 56.6%. Lean meat percentage objectively determined by EU referent dissection method was 51.9% in average.

Table 1

Results of descriptive statistics for warm carcass weight and measures used for lean meat percentage estimation of investigated pig carcasses

Measure	Method	Mean	St.dev.	Min	Max	N
Warm carcass weight (kg)		80.6	10.60	53	109	64
Fat thickness (mm)	Instrumental	17.5	6.40	6.80	38	60
Muscle thickness (mm)		53.9	10	32.60	72	60
Fat thickness (mm)	„Two points“	14.7	6.72	5	35	62
Muscle thicknes (mm)		68.4	6.77	50	80	62

Certain differences in lean meat percentage of investigated pig carcasses can be observed from previous table. In order to establish significance of differences between results of lean meat percentage estimation the data were statistically processed by GLM procedure of SAS 9.0 program package.

Table 2

Lean meat percentage of investigated pig carcasses

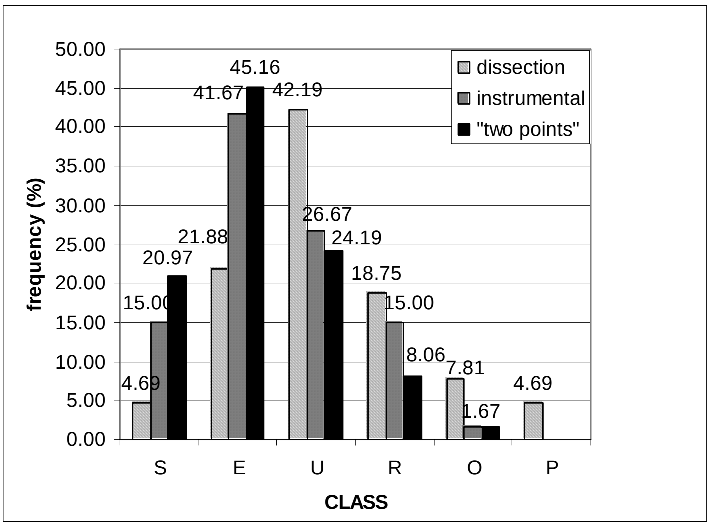
Method	„Two points"	Instrumental	Dissection
Mean	56.55[a]	55.13[a]	51.90[c]
Std. Dev.	5.028	4.919	5.780
Min. value	44.78	44.02	36.47
Max. value	68.24	66.75	65.54
N	62	60	64

Means within row with different superscript (a,c) differ at P<0,01.

From *Table 2* it can be observed that there were no statistically significant differences between lean meat percentage of pig carcasses estimated by TP method and instrumental method, but both estimations differed statistically ($P<0.01$) from lean meat percentage objectively determined by EU referent dissection. The methods for lean meat percentage evaluation obviously overestimated the meatiness of Croatian pig population and therefore there is a need to establish new coefficients in formulas for lean meat percentage assessment.

Figure 1

Relative distribution (%) of the pig carcasses into SEUROP quality classes



From *Figure 1* it can be observed that dissection, as the referent method, has classified only 4.69% of the pig carcasses into quality class S, whereas the methods for lean meat percentage estimation classified markedly more pig carcasses into this quality class (TP 20.97%, instrumental 15%). On the basis of dissection, the highest number of the pig carcasses was classified into quality class U (42.19%), in which TP method classified 24.19% and instrumental 26.67% of the pig carcasses. It can also be observed that according to dissected lean percentage, 4.69% of the pig carcasses fell into quality class P, while by other two methods none of the pig carcasses were classified into this class.

The presented results suggest that examined on-line lean percentage estimation methods prescribed in Croatia are not reliable for classification of pig carcasses into market classes any more.

CONCLUSION

On the basis of the investigation on comparison of different methods for lean percentage evaluation in pig carcasses, following can be concluded:

- Estimated lean percentage by TP and instrumental and methods differed statistically ($p<0.01$) from lean meat percentage objectively determined by EU referent dissection.
- There were no statistically significant differences between lean meat percentage of pig carcasses estimated by TP and instrumental method. This indicates that there was no significant influence of personnel who manages apparatus for measuring back fat and muscle thickness by instrumental method.
- Dissection, as the only objective and referent method, has classified only 4.69% of the pig carcasses into quality class S, whereas the other two methods of lean meat percentage estimation have classified markedly greater number of pig carcasses into this quality class (TP: 20.97%, instrumental: 15%). Dissection has classified 4.69% of the pig carcasses into quality class P while the other two methods have not classified any of the pig carcasses into this market class.
- The methods for evaluation of lean percentage overestimate the meatiness in the pig carcasses of the Croatian pig population.
- There is need to establish new coefficients in formulae for lean meat percentage assessment in Croatia.

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Carcass and tissues composition at Turopolje Pig Breed – Autochthonous Croatian Breed

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ABSTRACT

In Turopolje pig breed the carcass and tissues composition was established by analyzing the share of muscle (M), fat (F) and bone (B) tissues in the carcass and the chemical content (water, W, protein, P, lipid, L, ash, A) of the back fat and m. longissimus dorsi (MLD). Also, the some histochemical characteristics (diameter and proportion) of slow-twitch high-oxidative (SO), fast-twitch oxidative-glycolitic (FOG), and fast-twitch glycolitic (FG) fibre types of MLD were analyzed. Investigation was carried out on Turopolje breed pigs (n=10, age 679±20 days and 100.3 kg±4.9 kg). Pigs were fattened in the outdoor system of flood forests and marsh meadows biocenosis (Quercus robur and Deschampsietum caespitosae) according to traditional Croatian technology of low input feed (0.5 kg of corn seed/day/animal) in ecosystem. On the slaughter line the animals and carcasses were separately weighted and cut according to Weniger (1963) method and by total dissection. The samples of muscle and fat from the left side at the last rib level were taken after chilling 24 h at +4 °C and stored at -20 °C until chemical analysis. For histochemical analysis, sample from the same place and about 1 cm wide were taken 30 min after slaughter and frozen in liquid nitrogen until analysis. At Turopolje pig breed in the cold carcass (79.7 kg) were estimated the share of tissues respectively: M, 40.5%; F, 33.8%; B, 9.7% and share of the lard (4.0%) and double chain (3.2%). The chemical contents of MLD and back fat were respectively: W, 74.71% and 7.25; P 21.19% and 1.49; F, 1.46% and 91.76% and A, 1.06 and 0.06. The size and proportions of fibre types in MLD were respectively: SO, 38.9µm and 10.5%; FG, 57.7µm and 52.9%; FOG, 53.5µm and 36.7%.
(Keywords: Turopolje pig, carcass, tissue chemical content, fibre type)

INTRODUCTION

Turopolje pig breed is the autochthonous Croatian breed and one of older Europeans pigs and breeds (*Robić et al*., 1996). The numbers of scientific and expert papers were published about origin, historical and economic importance and factors which brought this breed into FAO list of endangered and disappearing breeds (*Loftus* and *Scherf*, 1993). This list was formed after signing the Convention on Biological Diversity (CBD) in Rio de Janeiro in June 1992. Republic of Croatia signed CBD (January 5, 1997) and in 1999. Croatia passes the strategy of biological diversity which includes Turopolje pig (*Radović*, 1999).

Table 1. gives the size of breeding population registered in herdbook of Turopolje pig breed in years 1996 (the start of programme of re-establishment and preservation) and in 2005 by annually reports of Croatian Livestock Center (*CLC*, 1997 and 2006).

Table 1

Breeding population of Turopolje pig breed in Croatia

Year	Sows	Boars	Gilt	Y.boar
1996	12	3	-	-
2005	129	14	107	-

Source: Annual report – pig breeding, CLC (1997, 2006).

Number of sows and boars (*Table 1*) besides the state subsidies, indicate the state of critical endangerment of this breed according to FAO standards (*Loftus* and *Scherf*, 1993), and during the last ten years the renewed is very slow, but number of gilts gives some the opportunity to change the present state. The breeding population is owned by family farms and by organization Universitas Communitas Nobilium Campi Turopolia (UCNCT, V. Gorica) which owns the majority of the population. It has to be said that UCNCT in 1996 started first with preservation by opening herdbook at CLC. This organization, a former land community (established in 13th century and legally suppressed in 1947, *Đikić et al*., 2002) renewed its activities and include them into the project of re-establishment and preservation of Turopolje pig as cultural and biological value as well as its natural habitat of origin and *in – situ* survival. It is important to emphasize that the traditional Croatian technology of low input pig production in the outdoor ecosystem of flood forests and marsh meadows, bound to Turopolje pig is a part of Croatian cultural heritage. Existing research results about characteristics of Turopolje pig mostly are published in monography "Turopolje pig – autochthonous Croatian breed – turopolka" (*Đikić et al.,* 2002).

However genetics conservation programs often focused only to maintain rare breeds, but many related questions need to be answered. Breeds are not genetically static. They are continuously developing and changing, and conservation policy must determine the historical point at which the true type existed. There are varieties and different types within a breed, which may have a risen as results of nature evolution or by introregression and the true type must be identified before conservation programs.
With this reason, the remainder of Turopolje pig population is under research of many biological traits, both on phenotypic and molecular level, and some of them are/were the problem at the first time (*Đikić et al*., 2002, 2006; *Harcet et al.*, 2006).

The objective of this study was to establish the carcass and tissues composition by analyzing the share of muscle, fat and bony tissues in the carcass, the chemical content of back fat and MLD as well as some characteristics of muscle fibre. Results of this research will be used as a base for defining the characteristics (standards) of today Turopolje pig breed, as well as a starting point for breeding and economical program of re-establishment, preservation and definition of production type of this breed.

MATERIALS AND METHODS

Animals and management

Investigation was carried out on Turopolje pigs (n=10). Pigs were fattened in the outdoor production system. The whole production cycle took place in the outdoor system of forest biocenosis (*Quercus robur, Fraxinus excelsior* and *Fagus sylvatica*) and marsh meadows (*Deschampsietum caespitosae*) in the Turopolje area (near to Zagreb). Traditional Croatian technology of low feed input (0.5 kg of corn seed/animal/day) in the ecosystem was implemented in the extensive management. Natural resources (acorn, soil, pasture) were utilized, but having a mind the environmental balance as well. No industrial feed, vitamins or mineral were used nor in piglets rearing neither in fattening. The average age of fattened pigs was 679±20 days.

Carcass and tissues compositions

In the abattoir for each pig the live weights (average 100.3±4.9 kg) and warm carcass weights (average 80.1±4.6 kg) were measured. After chilling through 24 hours at +4 °C the weights of cold carcass and the single of halves (left) for the dissection were recorded. The dissection of halves were performed according to *Weniger et al.* (1963) with the aim to determine the quantity of muscle (M), fat (with skin, F) bone (B) tissues and less valuable parts (LVP: head, lower parts of legs tail, kidney), lard (L) and double chain (DC) which were weighted separately. On the basis of mass of each tissue and the weight of halves the percentage of tissues in the carcass were determined.

For the chemical analysis the samples of muscle and fat tissues were taken from the MLD and belonging back fat (between 13/14 ribs) after chilling (24 h at +4°C). By chemical standard method in tissues were determined the percentage of water (W) protein (P) lipid (L) ash (A) and NET.

Muscle fibre characteristics were determined in the sample of the MLD and it was taken on dorsal side in the last rib level, 5 min after animal slaughtering and frozen in liquid nitrogen until analysis. By *Salomon* (1981) and *Pearse* (1972) methods, the histochemical differentiation of the three main fibre types, slow twitch oxidative, (SO) fast twitch glycolityc (FG) and fast twich oxidative-glycolytic (FOG) were obtained on the basis of the activity NADH, standard, alkaline and acid stabile adenosine tri-phosphatase (ATPase) and succinate dehydrogenase (SDH). The percentage of each fiber type was calculated from total number of fibres per cm^2 area. The fibre diameter was calculated from fibre cross section area assuming fibres were circular in shape.

The software package SAS (vs. 8, 1999) was used in data analyses. Experimental results were compared with literature (*Kralik* and *Petričević,* 2001; *Maltin et al.,* 1997 and others).

RESULTS AND DISSCUSION

Carcass composition

The results (*Table 2*) show cold carcass weights and composition of Turopolje pig breed and some production types (fatty type Mangalitsa and meat – fatty type Black Slavonian breeds and selected pigs) from literature (*Kralik* and *Petričević,* 2001; *Đikić* and *Jurić,* 2003).

According to the results (*Table 2*) of the Turopolje pig breed the established values of slaughtering and cold carcass weights in relation with age indicate very low daily gain at pigs produced in the outdoor system with technology of low feed input. Analysis of carcass composition (*Table 2*) in Turopolje breed showed that muscle: fat ratio in carcass without lard was in favor of muscle tissue. If both fat tissue and lard are included into the calculation then the ratio was 1.1:1. Regarding muscle: fat tissue relation in the carcass, according to other references (*Vukina*, 1961; *Belić et al.,* 1961) Turopolje pig is a late-mature fat production type of pig, together with Mangalitsa and Bagun. On the contrary, *Horvat* (1939) based on his own research conclude that fattened pigs with the average body weight of 101.7 kg and 81.6 kg of cold carcass weight were too fatty for fresh meat production and too little fatty for fat production (which was important at that time). Also the legs and shoulders conformations were very narrow and thin. The conclusions and results of Horvat induced us to investigate the tissue composition. However, if the established results (*Table 2*) for muscle:

fat tissue ratio were compared with recent data on breeds Mangalitsa and Black Slavonian (*Kralik* and *Petričević*, 2001) then the present population of Turopolje pig can be defined as a late mature combined meatiness – fatty type of pig for production in low feed input technology in the ecosystem of biocenosis of marsh meadows and flood forests. Besides, the obtained results (*Table 2*) indicate that Turopolje pig was not influenced by trends in pig selection directed by changes in demands for muscle and fat tissue on pig meat market which resulted in very high share of muscle tissue in carcass, (*Đikić* and *Jurić*, 2003). *Reeds et al.* (1993) reported that in the commercial fattened Landrace and Large White breeds at the age of 210 days and body weight of 90 kg, in the year 1940 muscle: fat tissue ratio was 0.87:1, while in 1980 it was 1:1. If these figures are compared to fattened Turopolje pigs, the status of selection according to carcass quality in the remaining population is visible.

Table 2

Carcass weight and composition

Breed/ Crossbred	**Carcass**	**Tissue (%)**			**LVP**	**L & DC**
	kg	**M**	**F**	**B**	**%**	**%**
	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD
Turopolje	79.7±4.4	40.5±1.39	33.8±1.29	9.7±0.74	8.8±0.84	4.0±0.65 3.2±0.82
Mangalitsa[A]	80.1±1.56	28.8±0.65	51.9±1.02	9.5±0.47	9.8±0.63	-
Black Slav[A]	79.5±2.41	32.4±1.31	48.4±1.57	9.9±0.84	9.3±0.79	-
SL[B]	79.0±4.59	49.2±3.42	27.9±4.1	10.4±0.76	9.2±0.59	3.3±0.90
HySL[B]	80.1±5.84	53.1±4.53	25.6±5.18	10.7±0.8	8.1±0.48	2.5±0.95
Hy[B]	78.6±5.16	55.3±3.11	23.9±4.32	11.4±0.84	7.7±0.58	1.7±0.65

Source:A = Kralik and Petričević. 2001; B = Đikić and Jurić. 2003.
M – muscle, F – fat, B – bone, LVP – less valuable parts, L – lard, DC – double chain, SL – Swedish landrace, Hy – hypor.

Tissues composition
Tissue composition of MLD and back fat are showed in the *Table 2* and *Table 3*.

Table 3

Chemical content of MLD and back fat (%)

Content (%)	**Tissue**	**Turopolje***	**Wild pig ****	**Mangalitsa****	**Black S. ****	**Hypor****
		$\bar{x}$ ±SD				
Water	M	74.71±0.56	74.40	70.14±0.90	70.36±0.76	73.12±1.01
	F	7.25±1.59		14.07±1.37	15.10±1.61	19.89±1.85
Protein	M	21.19±0.49	22.11	20.65±0.83	20.70±0.71	23.53±1.41
	F	1.49±0.48		4.39±0.73	4.60±0.71	5.03±0.66
Fat	M	1.46±0.35	1.55	8.21±1.44	7.86±1.09	2.23±0.13
	F	91.76±1.60		81.31±0.75	79.99±1.09	74.64±5.09
Ash	M	1.06±0.03	1.47	1.00±0.09	1.08±0.08	1.12±0.05
	F	0.06±0.03		0.23±0.07	0.31±0.08	0.44±0.14

*NET=MLD=±1.68±0.06; Fat=0.44±0.058. ***Source: Kralik* and *Petričević. 2001.*
M-muscle, F-fat.

The chemical content (*Table 3*) of the MLD indicated that meat of Turopolje breed pig contain low percentage of fat and high percentage of water, while proteins percentage are relatively high. The values of all components are very similar to the wild pig, but different in the comparison to the fatty type Mangalitsa and meaty – fatty Black Slavonian breeds. Deviations are in the content of water and fat. These indicate the specific way of the metabolism and storage of fat in the body and could be one of the reasons of the deviations in share of fat in comparison to other breeds (*Table 2*). Also, these results indicate the need of further of investigation of the other traits of tissues characteristics (iodine number, collagen content etc). Results in the table 4 show the muscle fiber characteristics of MLD of Turopolje pig and some crossbred pigs from literature.

Table 4

Muscle fibre characteristics of MLD

Breed/ crossbred	SO		FG		FOG	
	µm	%	µm	%	µm	%
	$\overline{x}$ ±SD					
Turopolje	38.87±12.06	10.5±1.09	57.7±14.83	52.9±14.86	53.5±14.57	36.7±18.27
LWSLP*	38.81±12.4	6.4±1.72	67.2±15.90	59.5±16.70	55.4±13.07	34.1±17.25
LWHyF**	45.18±4.84	11.74±2.99	53.2±4.38	58.1±3.53	48.84±4.75	30.18±2.46
DBM***	51.7±7.1	15.3±5.4	61.5±9.5	71.3±6.9	49.1±7.6	12.2±4.3

*Source: * LWSLP = (♂Large Whitex♀Swedish Landrace)x♂Pietrain (Đikić et al., 2006). **♂ Large Whitex♂ Hybrid Female.(Maltin et al., 1997). *** ♂Durocx♀Berlin Miniatur. (Fiedler et al., 2003).*

In Turopolje pig breed the fiber diameter and proportion of SO, FG and FOG showed and beside some higher variability's values in a normal range when compared to the general records for the swine as species by *Lawrie* (1998). However, the size of fiber types SO is more less in the Turopolje pig than in crossbred selected pigs LWHyF (average value of crossbred pigs of eight vary known breeding companies from Great Britain, *Maltin et al.,* 1997) and as wells as than DBM. But the diameter was similar to crossbred LWSL pigs. The established values for the size of FG and FOG fiber types in Turopolje pig breed were bigger than in LWHyF and smaller than in LWSL pigs. The differences in the percentages of each fiber types in composition of muscle and especially of FG in Turopolje pigs and crossbred pigs are visible.

The estimated results in the consideration with results of many authors cited by *Pas et al.* (2004) indicated that Turopolje pig breed could be genetically different compared to other breeds, if keep in mind the factors as well as its origin (*Ritzoffy*, 1931, 1933) and no intensive selection for lean muscle growth which in pigs may have caused, a large genetic change in fibre type composition. Investigation of carcass tissues composition need to continue and especially in the relation to the quality traits of meat.

CONCLUSIONS

Turopolje pig breed is in the state of critical endangerment (by FAO standard) but number of gilts suggests the change of that state.

In present population of Turopolje pig breed the some traits of carcass and tissue composition are specific and could be a consequence of specific historical conditions of breeding selection and production in the specific environment of the outdoor system.

The carcass and tissue composition give opportunity to setting up a program which would support reestablishment of the population on the economic base.

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Comparison of carcass and meat quality of purebred, F_1 and three-way crossbred pigs

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ABSTRACT

This study was performed on 88 pig carcasses evenly distributed into four groups regarding the origin: Large White (LW) and Swedish Landrace (SL) purebreds; LW×SL and (LW×SL) × Pi crossbred pigs. At approximately 100 kg live weight the pigs were slaughtered in one slaughter plant in eastern Croatia. In the slaughterhouse, carcass and meat quality traits were measured. It was found that three-way crossbred pigs had higher dissectional lean percentage (P<0.05) than both purebreds. Two-way crossbred pigs had the shortest carcass length. The highest ham circumference (P<0.05) had three-way crossbred pigs; these pigs had also significantly higher (P<0.05) MLD surface, compared to other pig groups. The highest fat surface and the most undesirable (P<0.05) fat/muscle ratio at MLD cut had LW purebred pigs which significantly differed (P<0.05) from other pig groups. Three-way crossbreds and SL purebreds had lower pH45 values than LW purebred and two-way crossbred pigs. LW purebreds had significantly higher pH24 value than other groups (P<0.05). Both purebred pigs had more favorable (P<0.05) WHC than three-way crosses; two-way crossbred pigs had intermediary values of this trait. Generally, three-way crossbred pigs with Piétrain as terminal sire had the leanest carcasses with the lowest meat quality.
(Keywords: pigs, carcass, meat quality, traits)

INTRODUCTION

Proper knowledge on the characteristics of breeds involved in the breeding program is essential to the decision making within the pork production and marketing systems (*Edwards et al.*, 2003). Croatian pork production is based on final hybrids of initial pig breeds included in the breeding program. The most used breeds within this program are Large White and Swedish Landrace for the production of F1 sows utilizing hybrid vigor in the aim of production of large litters characterized by advantageous fitness. On the side of the sire the most common used breed is Piétrain, ensuring the production of carcasses with high lean meat percentage. The production traits of final hybrids are strongly influenced by initial breeds used in program. Previous investigations proved that different breeds have a predetermined predisposition toward superiority in specific aspects of pork quality which may have inversed effect on other traits; e.g. involvement of Piétrain breed as terminal sire results in high lean production, but with reduced quality of meat (*Sonesson et al.*, 1998; *Edwards et al.*, 2003; *Šimek et al.*, 2003; *Kralik et al.*, 2004; *Kušec et al.*, 2004).

In that respect, the aim of present study is to investigate the carcass and meat quality traits of the breeds used most often in Croatian pig breeding program: Large

White (LW), Swedish Landrace (SL) purebreds; their F1 generation two-way crosses (LW×SL), and three-way crossbred pigs representing typical fattener pigs in Croatia; (LW×SL) × Pi (Piétrain).

MATERIALS AND METHODS

This study was performed on 88 carcasses of four pig groups (22 pigs in each group): 1st group=Large White (LW), 2nd group=Swedish Landrace (SL) purebreds; 3rd group LW x SL and the 4th group=(LW x SL) x Pi. The pigs were housed in the same conditions and fed the same diet during the fattening period. At the average age of 186 days and approximately 100 kg of live weight the pigs were slaughtered in one slaughter plant in eastern Croatia. At the slaughter line, the measurements of warm carcass weight, carcass length ("a" and "b"), ham length and circumference were taken from which ham index was calculated. Initial pH values (pH_i) were measured 45 minutes after the exsanguinations. The length of the carcass was measured from *os pubis* to the 1st rib (a) and from *os pubis* to *atlas* (b). The lean meat percentage (M%) in carcasses was obtained by "two points" and instrumental method according to Croatian Regulations (N.N. Nr. 119/1999) using the following formulas:

"Two points" method:

$$M\% = 47.978 + 26.0429\frac{F}{M} + 4.5154\sqrt{M} - 2.50181\log_{10} F - 8.4212\sqrt{F}$$

F: the minimum thickness of visible fat (including rind) on the midline of the split carcass in millimetres, covering the lumbar muscle (*M. glutaeus medius*),

M: the visible thickness of the lumbar muscle on the midline of the split carcass in millimetres, measured at the shortest connection between the front (cranial) end of the lumbar muscle and the upper (dorsal) edge of the vertebral canal.

Instrumental method:

$$M\% = 54.456-0.75027(F) + 0.21181(M)$$

F: the thickness of backfat (including rind) in millimetres, measured at 7 cm off the midline of the split carcass, between the second and third last ribs,

M: the thickness of the muscle in millimetres, measured at the same time and in the same place as F.

After 24 hours of cooling, cold carcass weight, backfat and loin eye area (cm^2), ultimate pH (pH_{24}) values, water holding capacity (w.h.c.) and color of *m. longissimus dorsi* were taken. The percentages of main tissues (muscle, fat and bones) were determined by total dissection of the carcasses by the method of *Weniger et al.* (1963) 24 hours after cooling. Backfat and muscle areas were measured by geometric procedure (*Comberg*, 1978) and expressed as the fat/loin eye area ratio; water holding capacity (w.h.c.) was determined using compression method by *Grau* and *Hamm* (1952). The color of the meat was measured photometrically by means of Göfo device (Göttingen) at *m. longissimus dorsi* cut, 24 hours after cooling.

RESULTS AND DISCUSSION

Carcass and meat quality traits of two different purebreds as well as of two- and three-way crossbred pigs were investigated in this study. Carcass traits of pigs originating from mentioned groups of pigs are presented on *Table 1*.

Table 1

Carcasses traits of investigated pigs

Indicator	Groups				P value
	1 (LW) $\overline{x} \pm s$	2 (SL) $\overline{x} \pm s$	3 (SL×LW) $\overline{x} \pm s$	4 (/LW×SL/×P) $\overline{x} \pm s$	
Weight of warm carcasses (kg)	83.09±3.56	81.05±5.66	80.50±10.12	81.06±5.13	0.550
Fat thickness by „TP“ method (mm)	19.83±8.04	18.40±4.98	19.62±5.42	16.94±5.04	0.362
Muscle thickness by „TP“ method (mm)	61.00±5,48	62.13±3,93	64.31±4,70	64.97±5,66	0.166
Lean meat percentage by „TP“ method (%)	51.75±4,74	52.46±3,58	51.98±3,24	53.93±4,07	0.298
Lean meat percentage by dissection (%)	52.61^{b}±3.27	53.25^{b}±3.25	55.27^{ab}±3.82	56.38^{a}±3.92	0.021
Lean meat percentage by instrumental method (%)	53.85±4.55	53.01±3.28	54.51±4.55	54.65±4.54	0.657
Carcass length – a (cm)	89.67^{a}±2.42	87.09^{ab}±4.61	84.59^{b}±5.75	87.76^{a}±4.38	0.045
Carcass length - b (cm)	104.33^{ab}±2.73	105.67^{a}±4.03	100.86^{b}±4.84	103.00^{ab}±4.74	0.019
Ham length (cm)	29.41±2.52	30.64±0.79	29.91±1.51	30.41±1.50	0.065
Ham circumference (cm)	69.55^{b}±1.41	68.50^{b}±2.76	69.86^{ab}±3.58	71.26^{a}±2.85	0.004
Ham index	$0.42±0.04^{a}$	$0.45±0.02^{b}$	$0.43±0.02^{a}$	$0.43±0.02^{a}$	0.011
MLD surface (cm^2)	33.76^{b}±4.64	35.59^{b}±5.67	37.46^{b}±6.04	41.84^{a}±7.50	<0.001
Surface of MLD belonging fat (cm^2)	24.81^{a}±5.87	18.66^{b}±4.25	20.40^{b}±7.15	19.74^{b}±4.73	0.002
Fat/MLD surface ratio	0.75^{a}±0,22	0.53^{b}±0,13	0.56^{b}±0,23	0.49^{b}±0.16	<0.001

Means within row with different superscript (a, b) differ at P<0.05.

Statistically significant differences between examined groups of pigs were established in lean percentage determined by dissection (P=0.021), carcass length “a” and “b” (P=0.045 and P=0.019, resp.), ham circumference and index (P=0.004 and P=0.011, resp.), MLD and belonging fat surface (P<0.001 and P=0.002, resp.), as well as fat/meat ratio at MLD cut (P<0.001).

Three-way crossbred pigs had higher lean percentage (P<0.05) determined by dissection, compared to purebreds. Second group consisted from F1 two-way crossbred pigs showed intermediary leanness and did not differ significantly from other examined groups of pigs. Third group (F1 crossbred pigs) had the shortest carcass length (length “a”=84.59 cm and length “b”=100.86 cm). The carcasses of pigs from this group were significantly shorter (P<0.05) from the 1st and 4th group (length “a”) and from second group (length “b”). The highest ham circumference had three-way crossbred pigs (71.26 cm) which statistically differed (P<0.05) from purebreds (69.55 and 68.50 cm); these pigs had also significantly higher (P<0.05) MLD surface, compared to other groups of investigated pigs. The highest fat surface at MLD cut (24.81 cm^2) had LW purebred pigs which significantly differed (P<0.05) from other pig groups; the same group of pigs also had the most undesirable (P<0.05) fat/muscle ratio at MLD cut.

Table 2 shows meat quality traits of pigs from investigated groups. From presented results, the strong influence (P<0.01) of breed on initial and ultimate pH values and water holding capacity (WHC) can be observed.

Table 2

Meat quality traits of investigated pigs

Indicator	Groups				P value
	1 (LW) $\overline{x} \pm s$	2 (SL) $\overline{x} \pm s$	3 (SLxLW) $\overline{x} \pm s$	4 (/LWxSL/xP) $\overline{x} \pm s$	
pH_{45}	6.32^{a}±0.37	6.04^{b}±0.35	6.29^{a}±0.27	5.96^{b}±0.34	<0.001
pH_{24}	5.84^{a}±0.23	5.72^{b}±0.21	5.73^{b}±0.19	5.63^{b}±0.17	0.003
W.H.C. (cm^2)	7.61^{b}±2.05	8.45^{b}±2.05	8.60^{ab}±1.85	9.52^{a}±1.73	0.004
Colour (Göfo value)	59.68±12.91	60.45±7.40	59.18±7.95	56.41±5.27	0.292

Means within row with different superscript (a, b) differ at P<0.05.

Higher values of pH45 were recorded for the pigs from the 1st and 3rd group (6.32 and 6.29, resp.) than for those from group 2 and 4 (6.04 and 5.96, resp.); the differences were statistically significant (P<0.01). Pigs from the 1st group had significantly higher pH24 value (5.84) than those from other groups (p<0.05). Purebred pigs (groups 1 and 2) had more favorable (P<0.05) WHC than three-way crosses. Two-way crossbred pigs had intermediary values measurements of this trait, and did not differ between the purebreds nor the three-way crossed pigs. No significant influence of the breed was found on the color of meat measured by Göfo device.

The value of pig carcasses increases with higher muscularity and lower fatness level, as pointed out by other authors (*Fisher et al.*, 2003; *Kolstad*, 2001). These authors also reported on difference between genotypes in mentioned characteristics which is supported by present study. The differences in lean meat percentage between the groups of investigated pigs regarding to methods of estimation (TP and instrumental) were not statistically significant, which could be expected since the differences in muscle and fat measures were insignificant as well. However, the differences in lean percentage between the groups of pigs were significant when carcasses were dissected into main tissues. This discrepancy between dissectional leanness and estimated lean percentage suggests that current methods for carcass classification of pigs at the slaughter line should be checked on accuracy. According to many authors, the meat quality decreases with the increase of carcass leanness and related traits (*Sonesson et al.*, 1998; *Kralik et al.*, 2001; *Kušec et al.*, 2004; *Šimek et al.*, 2004). In present study, three-way crossbred pigs with Piétrain as terminal sire line proved to have the leanest carcasses with obviously lower overall quality of meat which supports the findings of mentioned investigators.

CONCLUSION

The following conclusions can be drawn on the basis of present study:

Three-way crossbred pigs (SL×LW) x Pi had higher dissected lean percentage (P<0.05), compared to purebreds. Two-way crossbred pigs (SL×LW) had intermediary leanness and did not differ significantly from other examined groups of pigs (P>0.05). The differences between the groups of pigs in lean percentage estimated by “TP” and instrumental method were not statistically significant. The discrepancy between dissectional leanness and estimated lean percentage by both methods suggests that current methods for carcass classification of pigs at the slaughter line should be checked on accuracy of prediction.

F1 crossbred pigs (SL×LW) had the shortest carcass length which significantly differed (P<0.05) from the SL purebreds and three-way crossbred pigs (length "a") as well as from LW purebred pigs (length "b").

The highest ham circumference had three-way crossbred pigs (71.26 cm) which statistically differed (P<0.05) from purebreds (69.55 and 68.50 cm).

Fourth group of pigs (/SL×LW/ × Pi) had significantly higher (P<0.05) MLD surface, compared to other groups of investigated pigs; the highest fat surface at MLD cut (24.81 cm^2) had LW purebred pigs (P<0.05); the same pigs also had the most undesirable (P<0.05) fat/muscle ratio at MLD cut.

Statistically significant differences (P<0.01) were found in pH45 measurements between investigated groups of pigs; three-way crossbred pigs (/SL×LW/ × Pi) and SL purebreds had the lowest values of this trait (5.96 and 6.04, resp.) compared to LW purebred and SL×LW crossbred pigs (6.32 and 6.29, resp.).

Large White purebred pigs had significantly higher pH24 value (5.84) than those from other groups (p<0.05) while both purebred pigs (LW and SL) had more favorable (P<0.05) WHC than three-way crosses. In this respect, two-way crossbred pigs did not differ between the purebreds nor the three-way crossed pigs, having intermediary values measured for this trait.

It can be generally concluded that three-way crossbred pigs with Piétrain as terminal sire line had the leanest carcasses with the lowest overall quality of meat.

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Strategies of Hungarian pig-breeding farms

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ABSTRACT

The article analyses the basic strategic directions of the Hungarian pig-breeding plans, and determines a possible clustering of these farms. The majority of enterprises tries to increase the quality of production, aiming at optimalisation of the production technology and product-quality. Some enterprises try to differentiate their activity form concurrents by breeding of specific varieties. A third group of enterprises tries to increase their size by utilisation of relatively low input prices. Numerous enterprises try to stabilise their position by the minimalisation of input costs. In numerous cases there seems to be any well-defined strategy. Based on different productivity indicators the best results could be found in case of quality-oriented enterprises. The shrinking of production has improved the bargaining position of pig-breeders with feed suppliers, but there remained considerable problems with meat-industrial enterprises. As a consequence of worsening agricultural market situation and decreasing profit margin a further concentration process of producers can be predicted. At the same time, a general reconstruction of technical and technological infrastructure seems to be a necessity.
(Keywords: strategic planning, categorical principal component analysis, competitiveness)

INTRODUCTION

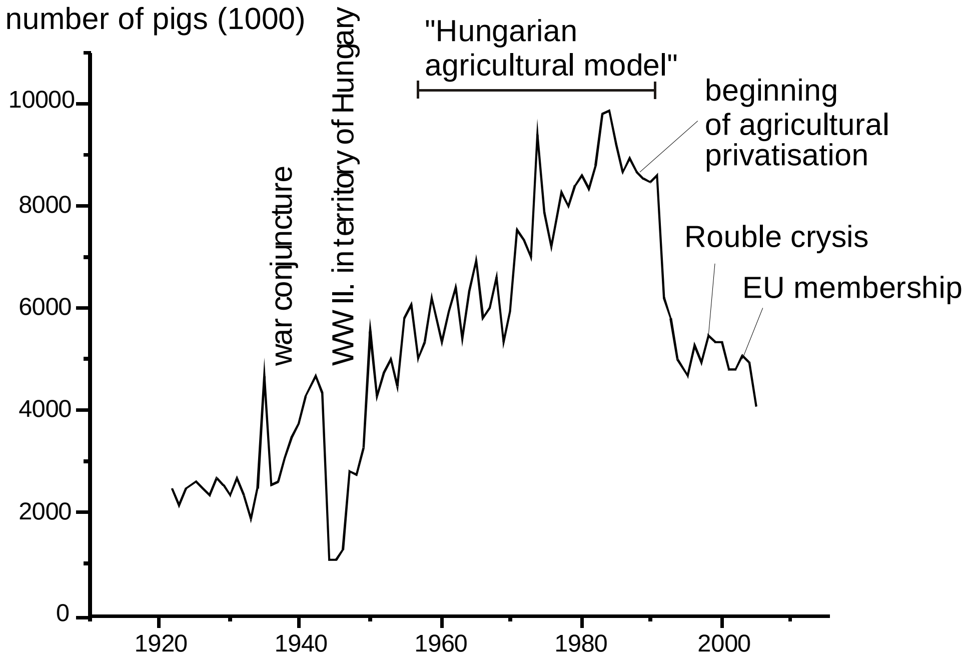
Pig production has been an emblematic part of the Hungarian agriculture (*Hajduné*, 1980). The demand of the former “socialist market” system meant a practically unlimited possibility for the increasing of meat production in the 70's and 80's of the last century. At this time the pig production has been divided into two sectors: the large–scale production of big farms in the ownership of co-operatives and state farms, and the small scale production (generally less than 20 weaners) in private (household) farms. These two types of activities were closely interrelated, because the small scale producers in most cases have been integrated by larger producers via contracts for supplying of veterinary service, forage, breeding sows and advisory service. As a result of these processes the Hungarian pig production has achieved a considerable increase from point of view of the quantity of production, but the technical and technological level and the efficiency of the agricultural production was relatively low even at the “golden age” of the Hungarian agriculture.

The Hungarian pig-breeding is especially sensitive to the changes in foreign markets (*Figure 1*). As a consequence of collapse of former foreign trade market structure, low level of competitiveness of Hungarian meat sector as well as the increasing input from another EU member states the Hungarian pig-production has decreased rather rapidly. These tendencies highlight the weak competitiveness of

Hungarian pig-breeding sector. Knowledge of the current tendencies and strategies of pig-breeding farms is a necessary precondition for the reconstruction of the sector.

Figure 1

The development of Hungarian pig-breeding (number of pigs in interval 1937-1944 convertet to the current territory of Hungary)



Source: own construction, based on Statistical Yearbooks of the Hungarian Central Statistical Office.

METHODOLOGY AND HYPOTHESIS DEVELOPMENT

The current survey is based on 150 (mostly anonymously) filled out questionnaires. To analyse the strategic directions of farms three focus group interviews have been carried out. The level of consent with directions have been measured by Likert-type interval scales, consisting of 1–5 scores, where 5 meat the highest level of consent.

H_1 The Hungarian pig-breeding enterprises can be divided into different from each other groups according to the strategic directions they follow.

H_2 The differences in strategies of enterprises are mirrored in their performance.

RESULTS

The have analysed the hidden structure of the responses by the categorical principal component analysis (*Table 1*).

For the determination of the exact number of the categories there is not a simple method available, that’s why we have utilised the generally utilised elbow-approach.

Based on this method four dimensions seemed to be satisfactory for the explanation of the variances.

Table 1

Results of the principal component analysis of the strategic ways of development of breeding

Strategic way of development	Number of dimensions			
	1	2	3	4
Utilisation of traditional, Hungarian pig breeds	0.452	0.914	0.214	0.264
Increasing of the forage-base of the plant by land –buying	0.324	0.657	0.788	0.341
Increasing of the professional knowledge of the blue-collar workers of the plant	0.874	0.314	0.224	0.784
Improvement of the quality of feeds	0.784	0.324	0.218	0.121
Utilisation of cheaper feed –purchasing resources	-0.756	0.121	0.224	0.911
Increasing of professional knowledge of the white-collar workers of the plant	0.741	0.214	0.211	0.478
Achievement of more favourable conditions with the feed suppliers	-0.234	0.354	0.354	0.847
Achievement of more favourable conditions with the meat industrial plants	0.844	0.547	0.145	0.441
Wider utilisation of the benchmarking for the evaluation of the plant	0.842	0.100	0.214	0.654
Improvement of the efficiency of veterinary medical attendance	0.377	0.125	0.128	0.214
Increasing of the programs for prevention	0.297	0.340	0.237	0.124
Bio-production	0.124	0.847	-0.421	.118
Improvement of the technology in framework of the current buildings	0.741	0.654	0.421	0.456
Increasing of the efficiency of the medical treatment	0.332	0.421	0.234	0.129
Improvement of the technology with the modernisation of the buildings	0.654	0.482	0.221	0.241
Utilisation of the western-European breeds	0.877	-0.658	0.241	0.235
Improvement of the feed conversion rate	0.715	0.232	0.652	0.148
Improvement of the meat categories	0.841	0.211	0.452	0.451
Improvement of the information and traacebility system of the farm	0.732	0.321	0.458	0.421
Increasing of the size of the herd to achieve a better	0.101	-0.587	0.847	0.154
Decreasing of the overhead cost of the plant	0.214	0.427	0.421	0.848

It is obvious, that in the first dimension the highest correlations are with the statements emphasising the production of specialities for the Hungarian meat industrial enterprises. It is worth to mention, that the price decreasing in this dimension has an extremely low weight. Put it in another way: this dimension is especially suitable for the differentiating of the strategic approaches into the direction of price lowering versus quality production.

In the second dimension the highest loading have statements, joining to the production of specific products. The distinctive competences in this dimension are the bio-production and the specific knowledge of workers. In a sense this approach is near to the strategic direction, summarised in first dimension. The only difference is that in the first dimension the highest loading have statements, joining to the quality of product in the traditional meat production system, in this, second dimension the most important distinctive competence is the quality increasing under modern market-conditions.

In the third dimension the highest loading have statements, joining to the increasing of the volume of the production. In this way a decreasing of unit cost and increasing of efficiency can be achieved.

In the fourth dimension the highest loading have the statements, joining to the cost-decreasing, by the better utilisation of the resources available. In this dimension the lowest loading had been given to statements, emphasising the product-differentiation. This is a well-defined strategy of large-scale pig breeders, with well-defined ways of development. Their owners are typically the meat-industrial plants or agricultural enterprises.

Based on the categorical principal component analysis, we have determined an object score for each respondent.

The scores obtained form the analyses above were utilised as the input variables to classify the pig-breeder plants. A five-cluster solution was found to maximise the distances between cluster means across four dimensions. To enhance the interpretation of each cluster, to each cluster has been given a fancy-name, emphasising the most important characteristic feature of the given cluster (*Table 2*).

- As a summary it can be stated, that the large-scale pig-breeding plants in Hungary can be divided into five groups, characteristically different from each other.
- In next phase of investigations we have analysed the hypothesis $H_{2.}$ The aim of this investigation has been to determine whether exist a direct relation between the strategies, the production potential and the performance of enterprises.
- It is obvious from *Table 3*, that there are characteristic differences between the farms, following differing ways of development.

CONCLUSIONS

Development of Hungarian pig-breeding sector should be built on pillars. These are as follows:

- Technological modernisation of pig-breeding plants. To achieve this, there is not enough money available in agricultural enterprises, that’s why the utilisation of outside resources gains in importance. The majority of pig-breeding plants did not get any additional support for the reconstruction or development of the breeding facilities from the EU budget or from the resources of the Hungarian Ministry of Agriculture and Rural Development. The pig-breeding plants investigated had only rather limited possibility for the access of bank credits. This can be explained on the supply side of the credits with the reluctance of banks to finance agricultural enterprises with low income-generating capacity.
- According to the latest results of the enterprise-theory, the enterprise should concentrate on the optimal utilisation of the core competences, outsourcing all of the activities, not forming an integral part of the basic activity of enterprise. In case of Hungarian pig breeders, however we can see a rather diversified field of activities. This phenomenon by itself is not a negative one, if it is a consequence of the well-established, predetermined strategy. A series of interviews of plant managers proves, that the majority of plants has not developed as a result of a well-determined strategy, rather a result of rather spontaneous events. That’s why a portfolio-cleaning should be essential.
- The regulatory frameworks should be more clear and transparent. In work of veterinarians, the advisory and controlling activities should be separated from each

other. The domestic pig production of pig-breeding plants workers should be prohibited.

- Continuous development of professional knowledge of white-and blue collar workers is essential. In this case there was an especially wide gap between the theory and practice, because during the last, rather turbulent years the education got only a secondary importance.
- In technological development of the plants the environmental and animal welfare considerations should be given an even higher priority too.

Table 2

The most important characteristic features of the pig-breeding farms investigated

Characteristic feature	The quality-oriented producers	Differen-tiators	Enlargers	Thrifts	Drifters
Most important strategic directions	Increasing of profitability by the quality increasing	Breeding traditional Hungarian varieties	Utilisation of economic of scale effect	Increasing of profitability by the decreasing of production cost	Decreasing the cost of production
Mode of the date of the beginning of operation	1985	1997	1971	1968	1974
Latest reconstruction	In last five years	It has not been necessary yet	In last ten years	In last ten years	In last ten years
Typical owner	Limited partnership. The majority of partnership interest is in hand of meat processing companies	Family farm, or limited partnership	Limited partnership	Co-operative with a high level of animal husbandry (>50% in turnover)	Co-operative with a low level in the animal husbandry (<50% in the turnover)
Typical variety	Holland	Mangalica (traditional Hungarian breed)	Hungarian large white pig	Kahyb, Hungarian large white pig	Kahyb, Hungahyb
Data and aim of variety-change	1999–2001 Increasing of efficiency	2001–2003 Back to traditional varieties	1999–2000 Increasing of efficiency	Not any variety-change	Not any variety-change
Utilisation of the capacity (in % of show-spaces)	60%	45%	70%	30%	35%
Share of E+S quality-grades according to the EUROP meat classification system in the output	71.2		62.1	63.4	64.2

Table 3

The productivity ratios of the pig-breeding plants

Indicator	The quality-oriented producers	Differen-tiators	Enlargers	Thrifts	Drifters
Fertility ration	81.5 (±7.2)	67.2 (±6.9)	75.5 (±8.4)	69.5 (±12.5)	66.4 (±7.2)
Live pigs born per litter	11.5 (±1.3)	7.2 (±1.9)	8.5 (±2.2)	7.9 (±2.8)	7.3 (±1.7)
Average number of litterings	2.4 (±0.3)	2.1 (±0.2)	2.3 (±0.3)	2.2 (±0.4)	2.3 (±0.3)
Mortality percentage to the age of 8 wks	8.1 (±2.3)	7.4 (±1.3)	10.4 (±2.3)	11.2 (±2.3)	11.6 (±2.3)
Pigs produced per year	25.4	17.2	22.4	21.5	16.2
Number of days of finishing	55	71	61	63	62

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The veterinary medicine in upgrading of competitiveness of Hungarian pig-production sector

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ABSTRACT

The Hungarian pig-breeding has achieved considerable results in 70's and 80's of the last century, but after the privatisation and the collapse of former integration structures a considerable differentiation begun between the pig-breeding plants. Based on a direct-question survey the article analyses some main features of the activities of veterinarians in large-scale Hungarian pig-breeding units in 2004. Under rather unfavourable economic conditions of pig-breeding farms in numerous cases the owners of these enterprises do not pay the sufficient attention to the prevention and the analysis of information, which could be obtained by systematic survey of veterinary status of farms. However, as it became obvious by analysing the structure of veterinary medication, the increasing attention to preventive veterinary medicine could contribute to the improvement of veterinary status.
(Keywords: swine, benchmarking, preventive medicine, veterinary strategy)

INTRODUCTION

The veterinary status exercises a direct effect on performance of swine farms. Contrary to the main trends of research in the EU states and the USA, the analysis of the micro-economic aspects of pig production, as well as the role of veterinary medicine in realization of strategic goals of enterprises are comparatively weekly developed in Hungary. While, e.g. in England, there are comprehensive works on the performance of pig-breeding (*Ridgeon*, 1993; *Robertson et al.*, 1991), in Hungary there are only some highly aggregated data on the cost-benefit relations and no quantitative information on effect of veterinary service on realization of strategic goals in pig production.

In market economies there are well-documented databases that emphasize the importance of quantifying the adverse economic effect of lack of preventive medicine. In his classic work, *Muirhead* (1987) has estimated the effects of various diseases on food conversion efficiency and days taken to reach 90 kg liveweight. The adverse effects in terms of both feed conversion efficiency (FCE) and growth rate are greater when a disease is introduced into a herd for the first time. This is illustrated in *Table 1*. The aim of current article is to determine some main features of veterinary practice in large-scale Hungarian pig farms.

MATERIALS AND METHODS

In framework of three focus-group interviews with experts working in pig farm management the basic directions of our questionnaire have been determined, aiming to reveal the position and strategy of Hungarian pig producers. In the second phase, a pilot study has been conducted to test the questionnaire. For practical reasons the original questionnaire have been divided into three parts: the general, comprehensive questionnaire inquiring the main strategic directions of the farms, which was sent to the farm managers, another questionnaire analysing the breeding technology, environmental management and the building engineering problems of units, which was sent to farm managers, and a specific questionnaire concerning the animal health management and animal welfare status of the farm, which was sent to the veterinary specialist.

Table 1

The effect of various diseases on food conversion efficiency and days taken to reach 90 kg liveweight

Disease	Reduced FCE	Increased days to 90 kg	Reduced FCE	Increased days to 90 kg
TGE	0.1	4–10	0–0.05	0–3
Epidemic diarrhoea	0.1	4–10	0	no data
Aujeszky’s disease	0.1–0.2	no data	0.1–0.2	6–14
Enzootic pneumonia	0.2–0.4	10–21	0.05–0.3	3–21
Haemophilus pneumonia	0.1–0.4	7–30	0.1–0.3	4–15
Atrophic rhinitis	0.1–0.2	4–15	0.1–0.2	4–15
Swine dysentery	0.05–0.2	15–20	0.05–0.1	4–8
Streptococcal meningitis	0.05	1–3	0.05	0
Mange	0.1–0.3	7–18	0.05–0.1	3–8
Internal parasites	0.1	7–18	0.1	3–6

Source: Muirhead, 1987.

Note 1. A deterioration in FCE of 0.1 is equivalent to a 3% increase in feed costs.

Note 2. Each extra day taken to grow from birth to slaughter at 90 kg liveweight is equivalent to reduce daily liveweight gain by 4 g.

After some improvement of the questionnaire, that was posted to more than 400 farms, representing approximately 90% of the large-scale pig farms in Hungary. 12% of the questionnaires were sent back, which could be increased up to 22.3% by a second call. In this way 103 questionnaires were acheived. Additional 47 questionnaires have been obtained by personal farm visits of the authors. The basic technical and technological indices of swine farms are summarized in *Table 2*. The survey was conducted from December 2003 to March 2004.

The survey on veterinary practice management, physical condition of buildings, breeding technology and veterinary status has been based on principles and suggestions of the current references (*Deen et al.,* 2001). Results of the basic technical and technological indices of swine farms are partly similar or equal to the findings of the survey done on commission of the Ministry of Agriculture and Rural Development in 2001 (*Ráki*, 2003).

RESULTS

Participation of veterinarians in the management of farms

In majority of cases (64%) the veterinarians are working as entrepreneurs at swine farms. The proportion of veterinarians among managers of swine farms is relatively high, 12%.

In other cases, the veterinarians are working as part- or (in some cases) as full-time employees.

In general, it can be stated, if the owner is a veterinarian, the number of weekly hours, spent on surveying herds is higher. This relationship has been proven by chi-square test (*Table 3*).

Table 2

Basic indices of the pig farms

Indices	Value and range
Average age of buildings	26.5 (±8.2) year
Average time from the latest reconstruction	5.3 (±4.8) year
Average number of sows in 2003	587.2 (±487)
Rate of fattening farms	42%
Rate of breeding farms	6%
Rate of mixed farms (for both breeding and fattening)	52%
Legal framework of the pig-breeding activity	
Family farm	12%
Private entrepreneurship	10%
Limited partnership	8%
Limited liability company	28%
Incorporated company	27%
Co-operative	15%

Table 3

Distribution of average weekly hours spent on surveying the herd*

Number of hours	Positive answers (%)
Less than 5 hours	14.3
5–10 hours	25.0
11–20 hours	38.6
21–30 hours	15.0
More than 30 hours	7.1

*Question: How many hours do you spend on surveying the herd?

It was positive, that the majority (88%) of respondents carries out regularly a post mortem examination; 40% of respondents dissects each fresh dead animal, except the sucking piglets. The scope of authority of veterinarians was rather diverse (*Table 4*).

Besides compulsory surveys the farms made regular diagnostic examinations (*Table 5*). It can be evaluated as a rather negative fact, that the majority of respondents does not have any record system for mortality statistics, neither in breeding, nor in fattening phase. This means, that they deprive themselves from numerous information, which could be very useful in strategic planning of different programs for preventive veterinary medicine, or in modernisation of technology and/or housing.

Table 4

The level of independence in decision-making of veterinarians

Statement	Positive answers (%)
Independent decision-making in problems influencing directly the veterinary status	100
I often make suggestions for conditions influencing indirectly the veterinary status of the herd (e.g. feeding technology, housing)	54
Independent decision-making in ordering medicaments (choosing supplier, cost of medication)	71
I have no independent decision-making right except in controlling infectious diseases	28

Table 5

The frequency of diagnostic examinations carried out beyond the compulsory tests

Frequency	Positive answers (%)
Monthly	41
Quarterly	47
Half-year	12

Evaluation of veterinary status from veterinarians' point of view

The majority (nearly two-third) of respondents stated that the current veterinary situation is not an efficient tool for a considerable development of efficiency in pig production. At the same time, 5% of respondents argued that the current veterinary status can be considered as a rather unfavourable one, and 29% of the veterinarians argued that the current veterinary status is acceptable, but there is room for considerable improvement. It is worth to emphasize that the rather negative opinion has been declared by veterinarians working in units of former cooperatives, where the question of ownership was not satisfactorily settled yet.

The next question tried to determine, what the most important obstacles to the improvement of veterinary status are by the respondents. The distribution of answers are summarised in *Table 6*.

Table 6

Causes of veterinary problems in the breeding units

Causes	Positive answers (%)
Lack of capital	73
Unfavourable epidemiological situation	15
Unskilled blue-collar workers	29
Neglectful attitude of managers	9
Undeveloped housing technology	55

According to the veterinary practitioners the most important obstacle to further development of veterinary status is the unfavourable housing technology. It can be explained by the fact, that the majority of animal breeding units has been built in the first years of 70's, following and adapting the most modern and sophisticated technological principles of that time, but, especially during the last two decades, there was not enough financial fund for the reconstruction of these plants.

Use of veterinary drugs

The use of medicaments shows a rather mixed picture. Within the total cost of drugs the antibiotics and vaccines have the highest share with considerable variance. In an explorative data analysis the boxes show the 50% (interquartile range) of values, and the mode do the thick lines (*Figure 1*).

Analysing the practice of choosing veterinary drugs a rather contradictory situation can be observed; on one hand the relatively high frequency of resistance tests can be evaluated as a positive characteristic, on the other hand the majority of veterinarians prefers the cheaper products (*Table 7*).

Figure 1

The share of different veterinary drugs within the total cost structure of veterinary pharmaceuticals

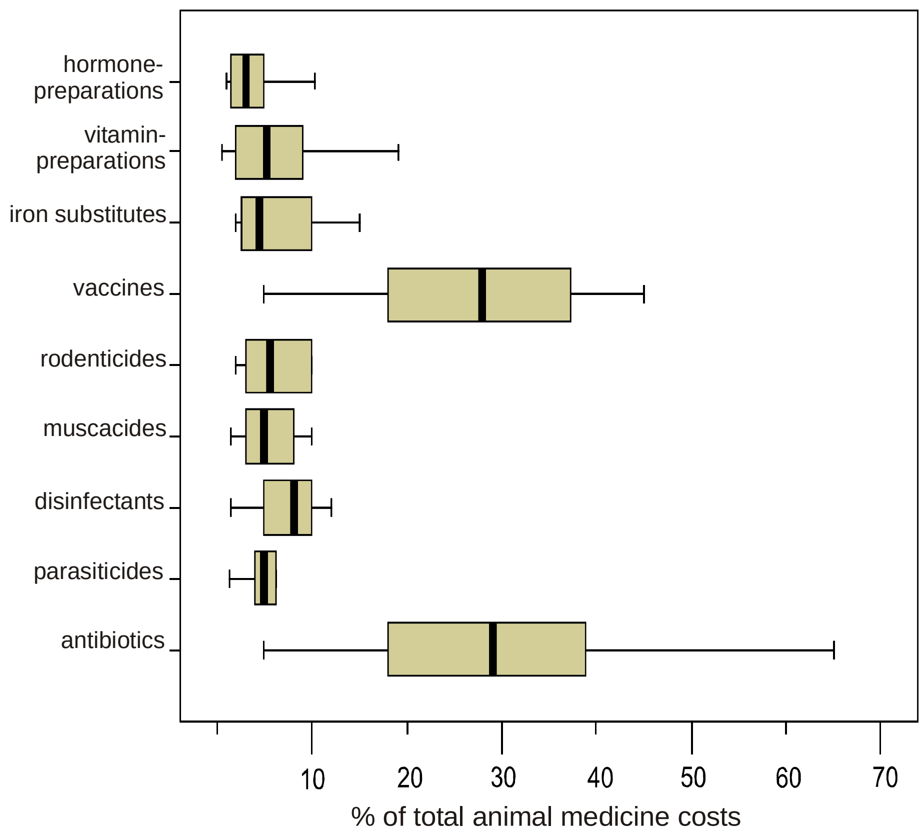

Table 7

Some features of attitudes and behaviour of veterinarians in choosing and using veterinary drugs

Statement	Positive answers (%)
I choose the antibiotics - at least partially by the resistance test	55
I choose the antibiotics by my former experiences	49
I choose the antibiotics on the basis of suggestions of advisors from pharmaceutical companies	61
When I choose a preparation, the most important factor is the price	59
When I choose a preparation, the origin of preparation (generic / original) has only a secondary importance	68
We often accomplish antibiotics/vaccine tests in our farm	14

The relatively low cost of medication per finishing pig could be explained by this thriftiness of veterinarians or owners (*Table 8*). However, this strategy seems to be a rather short-sighted one, because this could lead to a shift towards a more negative veterinary status of pig herds.

Table 8

Distribution of herds according to the cost of medication per finishing pig

Cost/finishing pig (Euro)	Distribution (%)
<2.0	53
2.1–4.0	31
4.1–6.0	9
>6.1	7

CONCLUSIONS

Based on our survey, it can be stated that the Hungarian pig-breeding is in a contradictory situation. There are large differences between the pig-breeding farms. As a consequence of agricultural transition and privatisation numerous new owners appeared in pig-breeding, often with any background knowledge on animal husbandry. These managers often try to follow a minimum cost strategy. As a consequence of that, they often neglect the veterinary considerations. That’s why the further education and post-gradual training courses should play an outstanding role to improve the collaboration between specialists with different background.

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SECTION 2

CATTLE BREEDING

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Analysis on crossbreeding in the Dutch dairy cattle population

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ABSTRACT

This study aimed to estimate heterosis for 305d-milk, fat and protein, and for calving interval in the Dutch dairy cattle population. Six breeds and 11 types of crosses were considered. A total of 5.913.654 and 3.679.183 primiparous cows were used for productive traits and for calving interval, respectively. Pedigree file was used to carry out the evolution by birth year (from 1976 to 2003) of the gene frequency in the Holstein Friesian (HF), Dutch Friesian (DF), and Mosa-Reno-Yssel (MRY) Dutch population. The effects included in the model of analysis, performed using software PEST, were: herd, year-season of calving, age at calving, lactation length, cross and random animal genetic effect. In the model for calving interval lactation length effect was replaced by 305d-milk yield. Means of 305d-milk, fat, and protein were high for HF and equal to 7300 kg, 315 kg, and 250 kg, respectively. Concerning calving interval, the best value was found for MRY (387 days). Non-additive genetic estimates for productive traits ranged from 0.8% (305d-fat) to 10.2% (305d-protein). Heterosis for calving interval appeared higher in crosses involving Brown Swiss (BS) and Montbéliarde (M) breeds, with values between -4.9% and -3.0%, corresponding to -12 and -21 days of calving interval, respectively. In conclusion, for yield traits higher percentages were provided by crosses in which Jersey (J) was present, while for calving interval combinations with BS performed better than others concerning heterosis. Future perspectives could consider the F1 following crossbred cows in order to estimate recombination losses.
(Keywords: crossbreeding, primiparous Dutch cows, heterosis, milk yield, calving interval)

INTRODUCTION

Crossbreeding is used widely in genetic improvement programs for many livestock species (*Weigel* and *Barlass*, 2003), in order to improve reproductive performances in female lines and productive characteristics in male ones, exploiting heterosis. This mating system provides a tool to increase health and efficiency in animals (*VanRaden* and *Sanders*, 2003), and a well-designed crossbreeding system allows the producer to combine the desirable characteristics of the breeds involved in the cross. Recently, crossbreeding has also become an interesting mating system in dairy cattle for many reasons. First, inbreeding level within each of the major dairy breeds is rapidly increasing, and crossbreeding may be an efficient way to cope with loss of genetic variation in dairy populations under selection, adding variability and reducing the impact of inbreeding depression (*Weigel* and *Barlass*, 2003). Second, direct payments for protein as well as fat in many milk pricing systems encourage some producers of the Holstein herds to consider crossbreeding as a tool to improve milk nutrient content, in order to enhance the ability of other breeds and breed crosses to compete with the Holsteins on an economic basis, especially in those countries where cheese industry is very important. Third, economic weight of functional traits such as reproductive efficiency, health and survival traits has risen in recent decades. Fourth, easy access to genetic material from almost anywhere in the world, strong competition among breeds like the Holstein, Brown Swiss and Jersey, and standardization of sire evaluations are making crossbreeding viable.

Some studies estimated non-additive genetic effects for milk yield traits and productive life in dairy cattle. Estimates of general heterosis in the USA conditions were 3.4%, 4.4%, and 4.1% for milk yield, fat yield, and protein yield, respectively, and 1.2% for productive life, a measure of longevity (*VanRaden* and *Sanders*, 2003). A Canadian study conducted on the Holstein×Ayrshire cross population (*McAllister et al*., 1994) provided heterosis estimates ranging from 16.5% to 20% for lifetime milk productive traits and estimates greater than 20% for the composite influence of these characteristics and growth, health, and reproductive traits on lifetime annualized discounted net returns (ADNR) (*McAllister*, 2002). In a French study on the Holstein×European Black and White cattle, heterosis reached 2 to 2.5% for yield traits (*Boichard et al*., 1993). Under New Zealand current market values for milk, *Lopez-Villalobos et al.* (2000) demonstrated the superior profitability of the rotational crossbred herds per hectare when compared to purebred ones.

This study aimed to describe the types of crossbreeding schemes currently performed in Dutch dairy cattle population, to analyze the evolution of the gene frequency in the male and female Dutch population, and to estimate heterosis for traits of economic relevance.

MATERIALS AND METHODS

Source of data and editing procedures

Heterosis was estimated in primiparous cows for 305d-milk, fat and protein yields, and for calving interval from the data recorded by NRS between 1980 and 2004. Breeds involved in this study were the Holstein Friesian (HF), Dutch Friesian (DF), Mosa-Reno-Yssel (MRY), Brown Swiss (BS), Montbéliarde (M) and Jersey (J); crossbreds studied were J×MRY, HF×J, DF×J, M×MRY, BS×DF, BS×MRY, BS×HF, DF×HF, DF×MRY, HF×M, and HF×MRY. A total of 6.973.735 data of milk yields distributed in 52.377 herds were available. Records with closed lactation shorter than 240 d or longer than 720 d, with missing sire or dam information, and with age at first calving shorter than 500 or longer than 1500 d were discarded prior to statistical analysis. In addition, for fertility data, calving intervals were restricted between 280 and 820 d.

After editing, yield trait data derived from 3,421,543 crossbreds and 2.492.111 purebreds reared in 39.915 herds, and calving interval data derived from 2.379.598 crossbred cows and 1.299.585 purebreds reared in 33.056 herds.

Pedigree file included 7.906.395 animals. All of them had sire and dam information. Pedigree data were used only for the main breeds (HF, DF, and MRY) in order to obtain gene proportion of each animal and to estimate the birth year variation of these genes.

Statistical analysis

Three classes of age at calving within breed (early, medium, tardive) and 3 classes of lactation length within breed (short, medium, long) were defined. Moreover, 3 classes of milk production levels (low, medium, high) were defined for calving interval.

Statistical analysis was performed using the PEST software (*Groeneveld et al*., 1990) according to a linear model considering the effects of herd, year-season of calving, age at calving, lactation length, cross and random animal genetic effect. All purebreds and crosses (F1 and following generations) were considered in the model of analysis. NRS provided (co)variance components used in this work. In the model for calving interval lactation length effect was replaced by 305d-milk yield.

Heterosis estimates

Production and reproduction traits solutions of crossbreds (F1) and purebreds were used for heterosis estimation.

RESULTS AND DISCUSSION

Data set composition used for production traits analysis (5.913.654 animals) is shown in *Table 1*. HF, MRY and DF represented the spreadest breeds in the Netherlands, with 21.9%, 10.8%, and 9.3% of the total sample, respectively.

Table 1

Number of purebred and crossbred primiparous cows for milk yield traits (n=5.913.654)

Main breed	Secondary breed					
	BS	**DF**	**HF**	**J**	**M**	**MRY**
BS	**192**	680	3049	26	5	2590
DF	9	**549.203**	768.431	1096	3	1737
HF	4885	1.634.618	**1.297.879**	7833	1895	534.681
J	21	1149	1360	**3152**	7	378
M	48	101	2598	2	**320**	1756
MRY	4678	18.155	428.243	679	830	**641.365**

Besides, crossbreds involving these breeds were the most common, especially HF×DF (27.6%), indicating a strong reference to Holstein Friesian in crossbreeding practice. Similar considerations can be done for the data set of calving interval (3.679.183 animals) that is shown in *Table 2*.

Table 2

Number of purebred and crossbred primiparous cows for calving interval (n=3.679.183)

Main breed	Secondary breed					
	BS	**DF**	**HF**	**J**	**M**	**MRY**
BS	**156**	527	2586	…	…	2253
DF	6	**21.052**	237.946	209	...	935
HF	4266	1.308.407	**1.097.073**	6464	1629	457.620
J	...	403	1022	**1885**	...	172
M	…	…	2160	…	**253**	1514
MRY	3625	9581	337.253	280	740	**179.166**

Descriptive statistics for traits studied are shown in *Table 3*. Means of 305d-milk, fat and protein were high for the HF breed, with 7300 kg, 315 kg, and 250 kg, respectively. Fair production levels resulted for BS and M, with values of 6100 kg for milk, 215 kg for protein, and 265 kg for fat. Concerning calving interval the MRY breed showed the lower value (387±55 days), while other breeds ranged between 398±69 (J) to 422±76 (BS) days. High values of calving interval could indicate the presence of fertility problems with a service period increase, that is probably due to the pregnancy difficulty.

Table 3

Descriptive statistics for 305d-milk yield, protein, fat, and calving interval in purebred primiparous cows

	Milk (kg)		Protein (kg)		Fat (kg)		Calving interval (d)	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
BS	6078	1101	220	42	273	53	422	76
DF	4903	823	165	28	208	36	403	63
HF	7322	1228	250	40	315	50	410	73
J	4105	826	166	31	257	44	398	69
M	6059	1027	210	34	256	40	407	75
MRY	4964	822	172	29	209	37	387	55

The variation per birth year of the HF, DF, and MRY gene percentage in female and male populations are shown in *Figure 1* and *2*. The HF genes increased rapidly between 1970s and 1990s from 15% to more than 90%. This variation in the Europe countries was caused to an important introduction of USA genetic materials. Conversely, in the same years the fraction of DF genes decreased in male as well as in female population, passed from high (70%) to small values (5%). In the 1970s the Holstein male population resulted in a major fraction of genes if compared to female one, probably due to the initial importation of Holstein bulls as live calves or embryos in the Europe.

Figure 1

Distribution per birth year of female genes in the HF, DF, and MRY dairy population

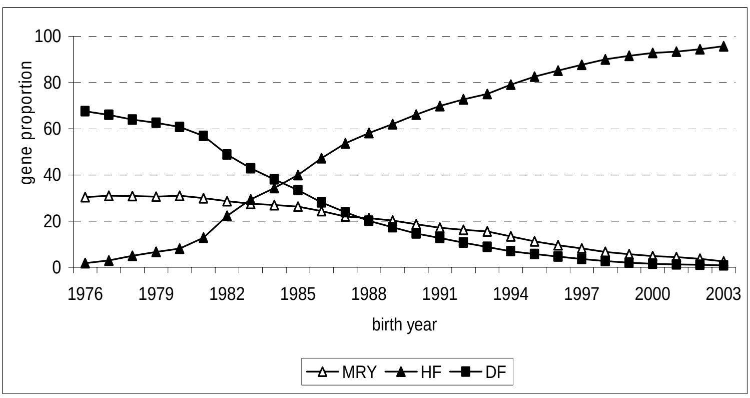
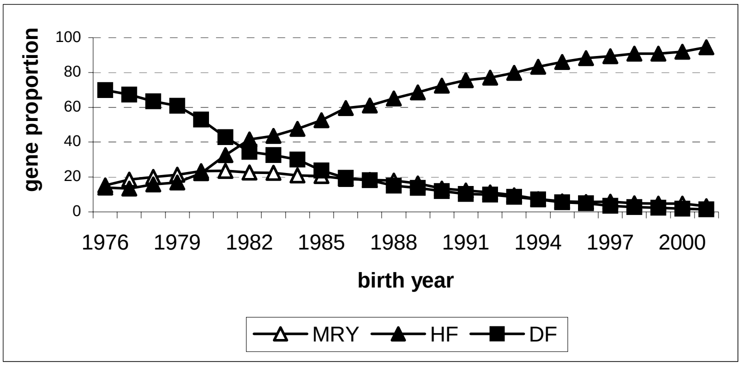


Figure 2

Distribution per birth year of male genes in the HF, DF, and MRY dairy population



Non-additive genetic estimates for yield traits and calving interval for F1 crosses, with at least 100 observations, are shown in *Table 4*. The solutions for 305d-milk yield ranged from 9.8% heterosis for J×MRY to 1.2% for DF×MRY. The solutions for 305d-protein yield ranged from 10.2% for J×MRY to 1.5% for DF×MRY. The solutions for 305d-fat yield ranged from 9.1% for J×MRY to 0.8% for DF×MRY. Finally, calving interval heterosis was higher in the combinations where BS and M were present, with percentage values ranged between -4.9% to -3.0%, corresponding to -12 and -21 days, respectively. Means of heterosis for productive traits were similar to those found by *VanRaden* and *Sanders* (2003), and higher percentages were provided by crosses in which Jersey was present.

Table 4

Heterosis estimates for milk yield traits and calving interval

	n.	305d-Milk		305d-Protein		305d-Fat		n.	Calving interval	
		H (kg)	H (%)	H (kg)	H (%)	H (kg)	H (%)		H (d)	H (%)
J×MRY	402	515	9.8	19	10.2	23.0	9.1	110	-6	-1.4
HF×J	3109	376	6.8	16	8.2	23.0	8.8	2549	-6	-1.4
DF×J	1001	503	9.5	16	8.5	23.0	8.9	160	-6	-1.4
M×MRY	508	250	4.3	9	4.5	14.0	5.4	452	-12	-3.0
BS×DF	186	385	6.7	11	5.4	14.5	5.7	115	-13	-3.1
BS×MRY	3004	378	6.6	10	5.0	14.5	5.8	2153	-16	-3.8
BS×HF	1311	241	4.0	6	2.9	11.0	4.2	1086	-21	-4.9
DF×HF	598.100	137	2.3	5	2.4	8.0	2.8	187.448	-3	-0.8
DF×MRY	7759	69	1.2	3	1.5	2.0	0.8	2338	-5	-1.3
HF×M	766	119	2.0	6	2.9	9.0	3.4	621	-14	-3.5
HF×MRY	218.337	131	2.2	5	2.4	8.0	2.9	163.889	-3	-0.8

CONCLUSIONS

HF, DF, and MRY resulted the spreadest breeds in the Netherlands, both as purebred and crossbred, while the others were less represented. In the last 30 years there was an increase of genes fraction for the HF breed, and a decrease for MRY and DF breeds.
Regarding calving interval values found for crosses involving BS were interesting with heterosis estimates ranged between -4.9% to -3.1%.

This paper was a preliminary analysis of non-additive effects due to crossbreeding. Future studies will be led to estimate maternal effects of traits studied in this work, and to estimate recombination losses in the F1 following generations. Moreover, heterosis of other functional and sanitary traits (e.g. SCC) could be considered in future perspectives.

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Genetic evaluation of milking speed for Slovenian Holstein cattle regarding to different scoring approaches

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ABSTRACT

The objective of this research was to determine differences between estimated variance components and heritability of milking speed for Slovenian Holstein cattle regarding to different scoring approaches. Milking speed was scored by two different scoring approaches. From year 1989 to 2004, milking speed was scored on scale of 1–3, while from year 2004, scale of 1–5 was applied in scoring of milking speed. For analysis, from both approaches, 71.757 records were available. For genetic evaluation of milking speed univariate model and multi-trait model was used. In univariate model milking speed data on scale of 1–3 was evaluated, while in multi-trait model, both, milking speed data on scale of 1–3 as one trait, and milking speed data on scale of 1–5 as second trait, was evaluated. Herd and residual variances were lower in multi-trait model in comparison with variances in univariate model, while genetic variance was higher in multi-trait model. Variances for all random effects in multi-trait model were higher for new trait (MS 5) than for old trait (MS 3). Heritability for MS 5 was considerably higher than heritability for MS 3 estimated from both models. Breeding values for MS 5 had almost three time greater standard deviation in relation to breeding values for MS 3 in multi-trait. Appliance of new scoring approach resulted in genetic variance increase, which finally resulted in higher heritability value, which would enable faster selection response. Further investigation based on revalorisation of economic value for milking speed in calculation of Total Merit Index is needed.
(Keywords: genetic evaluation, milking speed, scoring approaches, cattle)

INTRODUCTION

Milking speed of dairy cows is economically important trait. Dairy cows with longer period of milking duration require more time and energy for milking. However, to fast milking speed potentially increase possibility for development of udder diseases that could finally result in culling. Milking speed could be measured with a LactoCorder within official Milk Recording, when data are recorded as average milking speed in kg per minute, or scored on scale 1–3 and 1–5 within Linear Scoring (*Rensing* and *Ruten*, 2005). Heritability for milking speed scored on scale 1–5 for Canadian Holstein was 0.20 (*Blair*, 2003). *Rensing* and *Ruten* (2005) reported that heritability for German Holstein for measured milking speed was 0.28, while for owners scored milking speed heritability was 0.10. The objective of this research was to determine differences between estimated variance components and heritability of milking speed for Slovenian Holstein cattle regarding to different scoring approaches.

MATERIALS AND METHODS

Milking speed was scored by two different scoring approaches. From year 1989 to 2004, milking speed was recorded within Linear Scoring of first lactation cows. The linear scoring expert asked the owner to score milking speed on scale of 1–3 (where: 1–slow, 2–average, 3–fast). From this scoring approach, 51.111 records were available for genetic evaluation. From year 2004, scale of 1–5 (where: 1–very slow, 2–slow, 3–average, 4–fast, 5–very fast) was applied in scoring of milking speed. Data on milking speed were recorded within Milk Recording of first lactation cows. For genetic evaluation, from this approach, 20.646 records were available. Data used for evaluation were from Central data base of Agricultural institute of Slovenia. Descriptive statistics for milking speed are reported in *Table 1* for old data and in *Table 2* for new data.

Table 1

Descriptive statistics for data collected from 1989 to 2004

trait	n	mean	SD	CV	min	max
milking speed (3)	51.111	2.227	0.478	21.453	1.000	3.000
age at calving (days)	51.111	863.89	106.67	12.35	506	1.200
age at scoring (days)	51.111	1.021.19	124.67	12.20	588	1.497
period from calving to scoring	51.111	157.29	64.22	40.83	5	365

Table 2

Descriptive statistics for data collected from 2004 to 2005

trait	n	mean	SD	CV	min	max
milking speed (5)	20,646	3.744	0.767	20.483	1.000	5.000
age at calving (days)	20,646	857.78	109.59	12.78	488	1200
age at scoring (days)	20,646	927.81	112.02	12.07	563	1426
period from calving to scoring	20,646	70.03	23.64	33.75	5	326

Descriptive statistic show that mean values for milking speed are in booth cases over expected theoretical mean value. The age of first calving is not very different in comparison of this parameter in both system of scoring. In new system the cows are just six days younger at first calving. One of main things in case of changes of scoring system is also differences in period from calving to scoring. In old system the linear scoring expert goes to farm in main cases just twice per year and for this reason the mean value for period from calving to scoring is nearly half year. In new system of scoring milking speed the mean value of period from calving to scoring is more than twice smaller in comparison with this parameter in old system. This change we can explain with scoring within Milk Recording in most cases at second and third milk control.

According to age at first calving, cows were divided into five groups, S1 (<750 days), S2 (750–810 days), S3 (810–870 days), S4 (870–930 days), S5 (>930 days). Additionally, according to period from calving to scoring, cows were divided into five groups, D1 (<60 days), D2 (60–90 days), D3 (90–120 days), D4 (120–150 days), D5 (>150 days). For genetic evaluation of milking speed univariate model and multi-trait model was used. In univariate model milking speed data on scale of 1–3 was evaluated, while in multi-trait model, both, milking speed data on scale of 1–3 as one trait, and milking speed data on scale of 1–5 as second trait, was evaluated.

The statistical model for milking speed was the same in all cases:

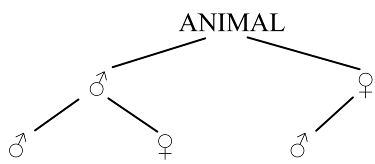
$$y_{ijkl} = OL_i + T_j + SD_k + h_l + a_{ijkl} + e_{ijkl} \quad (1)$$

where:
y_{ijkl} – records of milking speed (on scale of 1–3 or on scale of 1–5) assumed normally distributed,
OL_i – concatenated effects of expert and year of scoring,
T_j – calving season,
SD_k – concatenated effects of classes of age at first calving and classes of period from calving to scoring,
h_l – random effect of herd,
a_{ijkl} – random additive genetic effect of animal,
e_{ijkl} – random residual effect.

In covariance estimation relationship structure as shown in *Figure 1* was used. Animal, shown on *Figure 1*, presents each first calving cow with score of milking speed.

Figure 1

Relationship structure



For statistical analysis the SAS/STAT package was used (*SAS Institute Inc*., 2000), while for estimation of variance components and heritabilities MTC program (Fortran) was used. For breeding values estimation MTJAAM program (Fortran) was used.

RESULTS AND DISCUSSION

As it was expected, based on material analysis, differences in estimated dispersion parameters between models were considerable (*Table 3*). Residual variance, in univariate model, was considerably higher in comparison with genetic and herd variances. Relationship between those variances resulted in low heritability, which in case of univariate model was only 0.026 (*Table 4*). Herd and residual variances were lower in multi-trait model in comparison with variances in univariate model, while genetic variance was higher in multi-trait model (*Table 3*). Variances for all random effects in multi-trait model were higher for new trait (MS 5) than for old trait (MS 3). In all cases covariances were low which means that correlation between traits was close to zero.

Due to differences between variances according to models, difference between heritabilities was also expected. Although covariances between new and old trait were low, heritability for MS 3 in multi-trait model considerably increased in relation to heritability for MS 3 in univariate model. Heritability for milking speed scored on scale of 1–5 (MS 5) was considerably higher than heritability for milking speed scored on scale 1–3 (MS 3) estimated from both models (*Table 4*). Similar results of heritability value for milking speed scored on scale 1–5 for Canadian Holstein were obtained by *Blair* (2003), while *Erf et al.* (1992) and *Boettcher et al*. (1998) reported considerably lower heritability.

Table 3

Variance and covariance components for random effects

model	trait			variance
univariate model			herd	0.008275
			animal	0.002895
			residual	0.100233
			MS (5)	MS (3)
multi-trait model	herd	MS (5)	0.006037	0.000365
		MS (3)	0.000365	0.004541
	animal	MS (5)	0.035060	0.000724
		MS (3)	0.000724	0.009179
	residual	MS (5)	0.096899	-0.001232
		MS (3)	-0.001232	0.067850

Table 4

Heritabilities according to models

model	trait	heritability
univariate model	milking speed (3)	0.026
multi-trait model	milking speed (3)	0.116
	milking speed (5)	0.254

Figure 2 and *3* show relations between standardised breeding values estimated on old way of scoring and breeding values estimated with multi-trait model. With purpose of standardisation of breeding values standard deviation of 12 point and mean value of 100 point were used. In case of breeding value for milking speed standard deviations of breeding values were: 0.0201 for old MS, 0.0363 for new MS 3 and 0.0978 for new MS 5. This results shown us that breeding values were more variable in multi-trait model. Higher variability of breeding values for MS 3 in multi-trait model in relation to breeding values for MS 3 in univariate model indicate that new scoring approach (scale 1–5) induce higher variability.

Breeding values for MS 5 had almost three time greater standard deviation in relation to breeding values for MS 3 in multi-trait. Although the differences in variability of breeding values for milking speed scored on scale 1–3 were relatively high, the differences between rang of animals according to breeding value between univariate and multi-trait model were small (*Figure 2*).

Relationship between old breeding values and breeding values estimated with multi-trait model for milking speed scored on scale 1–5 was negligible (*Figure 3*) which means that, from statistical point of view, MS 5 and MS 3 are different traits, despite of that, from biological point of view MS 5 and MS 3 are same trait.

Figure 2

Relation between old breeding value and new breeding value estimated with multi-trait model for MS 3

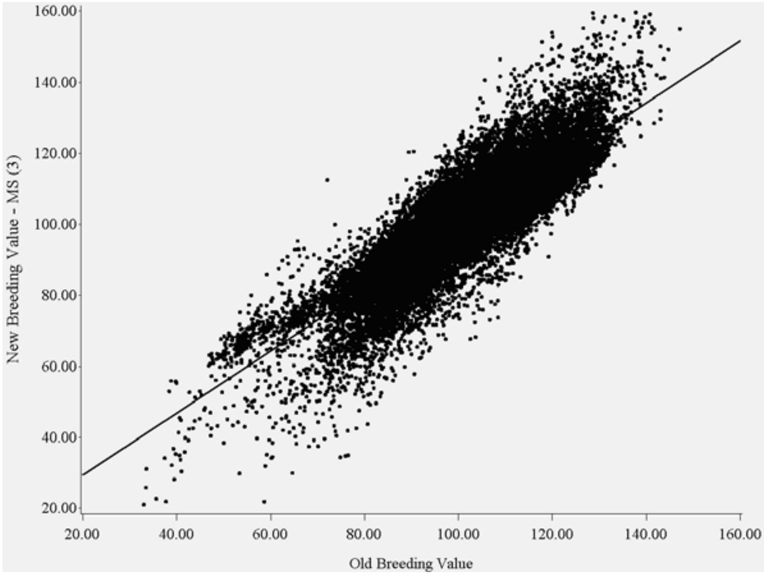
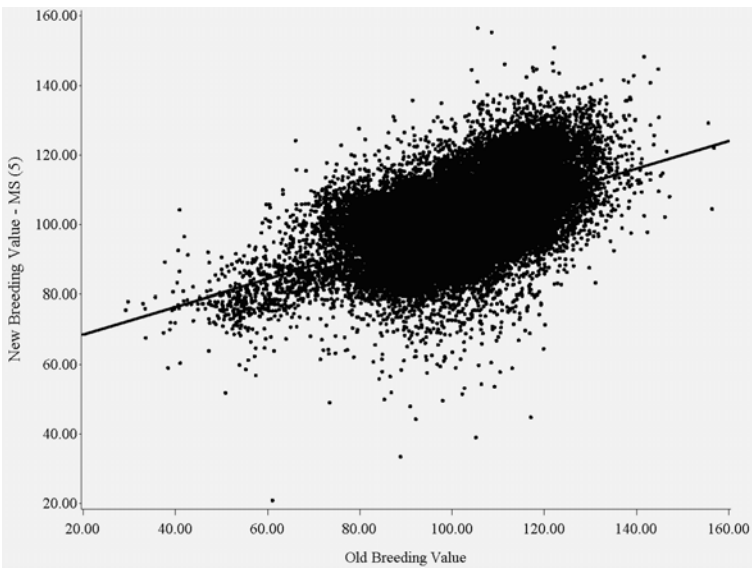


Figure 3

Relation between old breeding value and new breeding value estimated with multi-trait model for MS 5



CONCLUSIONS

With introduction of new scoring approach for milking speed in year 2004, changes in results were expected. Changes in estimated breeding values were also expected. Appliance of new scoring approach resulted in genetic variance increase, which finally resulted in higher heritability value. Higher heritability values enables faster selection response. So far, economic value for milking speed in calculation of Total Merit Index was 0.03, but selection for mentioned trait was not successful because of low heritability value. Therefore, further investigation should be based on revalorisation of economic value for milking speed in calculation of Total Merit Index.

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Various approaches to daily milk yield prediction from alternative milk recording scheme

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ABSTRACT

The objective of this research was to compare different approaches to daily milk yield prediction from alternative milk recording scheme (single morning and evening milking records). The data used in this study were 3.730 individual test-day milk yield records collected from November 2004 to November 2005 on 560 cows reared on 15 family farms in Croatia. Daily milk yield, as well as, daily fat and protein content were predicted by several different approaches. The correlations between true and estimated daily milk yield are slightly lower when prediction is based on evening milkings, while the correlations between true and estimated daily fat as well as protein content are slightly higher when prediction is based on evening milkings. Model D, which included single yields as covariate as well as effect of daily interval, and model E, which also included effect of lactation stage as lactation curve by Ali and Schaeffer, gives the best fit to the data both for prediction of daily milk yield or milk content (fat and protein) based on morning or evening milkings. Differences between those two models were minor and statistically insignificant, so we would recommend use of model D in practice as the model which could be easier to implement in routine work.
(Keywords: alternative milk recording scheme, daily milk yield, prediction, cows)

INTRODUCTION

Milk recording provides data acquisition on milk yield which are necessary for genetic evaluation and herd management of dairy animals. Numerous milk recording schemes have been developed in many countries in the last decades (*Porzio*, 1953; *McDaniel*, 1969; *Wiggans*, 1981) with purpose of supplementation of the standard four-weekly testing scheme (A4) which is considered as the most expensive one. The alternative milk recording (morning or evening) testing scheme was designed to gain lower cost and to retain reasonable accuracy in daily milk yields prediction. The accuracy of daily milk yield prediction is the most important factor in alternative milk recording scheme. With aim to predict daily milk yield from single milking weights various models have been developed. Depending on the model, different factors that influence milk production were taken into account, like breed, parity, lactation stage, and the interval between successive milkings (*Hargrove*, 1994; *Cassandro et al*., 1995; *Klopčič*, 2004). The milking interval is the most important factor when daily milk yield is predicting from morning or evening milkings. The objective of this research was to compare different approaches to daily milk yield prediction from alternative milk recording scheme (single morning and evening milking records).

MATERIALS AND METHODS

The data used in this study were 3.730 individual test-day milk yield records collected from November 2004 to November 2005 on 560 cows reared on 15 family farms in Croatia. At each recording, milk yield was measured in the evening and in the morning. Daily milk yield was computed as evening plus morning measured yield. Also, at each milking, initial time of current milking and initial time of previous milking for each animal was registered. The interval between successive milkings was computed as the time from the beginning of previous milking to the beginning of current milking. For analysis of milk composition three samples were taken from each cow: one sample at each milking (evening and morning) and one proportional milk sample. Logical control of data was performed according to *ICAR* standards (2003). Additionally, a linear regression of daily to evening or morning records was fitted in order to detect outliers. Residuals over three standard deviations were taken as outliers and deleted from data set. Variability of daily, morning and evening milk yield, fat and protein content as well as daily and nightly interval between successive milkings are reported in *Table 1*.

Table 1

Descriptive statistics for milk traits (n=3.730)

Trait	Milk yield, kg			Fat content, %			Protein content, %		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
Daily	19.79	6.39	32.28	4.34	0.81	18.65	3.50	0.43	12.42
Morning	10.51	3.56	33.88	4.21	0.85	20.24	3.48	0.44	12.63
Evening	9.26	3.11	33.53	4.45	0.93	20.85	3.52	0.45	12.73
Nightly interval, min	766.00	54.92	7.17						
Daily interval, min	676.93	54.56	8.06						

Correlations between daily, morning or evening milk yield as well as fat and protein content are shown in *Table 2.* It is evident that evening milkings have lower correlations with daily yields than morning milkings which is in agreement with published results (*Lee and Wardrop*, 1984; *Cassandro et al*., 1995; *Trappmann et al*., 1998; *Liu et al*., 2000).

Table 2

Correlations between daily, morning or evening milk yield, fat and protein content

Trait	daily – morning		daily – evening		morning – evening	
	r	p	r	p	r	p
Milk yield, kg	0.965	<0,0001	0.953	<0,0001	0.841	<0,0001
Fat content, %	0.893	<0,0001	0.911	<0,0001	0.628	<0,0001
Protein content, %	0.972	<0,0001	0.974	<0,0001	0.894	<0,0001

If milk composition is taken into consideration, both, the correlation between daily and morning fat content and correlation between daily and morning protein content are lower than correlation among daily and evening contents. The similar results were reported by *Klopčič* (2004). The lowest correlation with its daily measurements has fat content measured on single milkings, which means that the accuracy of daily yield prediction from single records will be lowest if prediction of fat content is observed.

Table 3

Selected approaches to daily milk yield (fat and protein content) prediction

model	statistical model
A	$y_i = 2m_i$; ($y_i = m_i$)
B	$y_i = \mu + b_1 m_i + e_i$
C	$y_{ij} = \mu_j + b_{1j} m_{ij} + b_{2j} \left(d_{ij} - 158\right)^*$
D	$y_i = \mu + b_1 m_i + b_2 t_i + e_i$
E	$y_i = \mu + b_1 m_i + b_2 t_i + b_8 (d_i / 305) + b_9 (d_i / 305)^2 + b_{10} \ln(305 / d_i) + b_{11} \ln^2 (305 / d_i) + e_i$

y: daily yield, μ: intercept, m: evening or morning yield (content), t: interval between successive milkings, d: lactation stage (days), e: residual, *modified DeLorenzo and Wiggans' model, each milking interval classes *j* has one regression (*DeLorenzo* and *Wiggans*, 1986).

For statistical analysis the SAS/STAT package was used (*SAS Institute Inc*., 2000). Daily milk yield and daily fat content were predicted by five (A, B, C, D, E) different approaches, while the daily protein content was predicted using four (A, B, D, E) different approaches (*Table 3*). Different approaches to daily milk yield prediction from alternative milk recording scheme were compared on the basis of the correlation between true and estimated daily milk yields (r), bias (mean difference between estimated and true yields) and accuracy (standard deviation of the difference between estimated and true yields).

RESULTS AND DISCUSSION

Table 4 shows correlations between true and estimated daily milk yields, as well as bias and accuracy of different approaches to daily milk yield prediction from morning or evening milkings. The model with the highest correlation and lowest bias fits the best to the data set. The correlation enhances with the complexity of the models which means that the most complex model, model E, gives the best fit to the data both for prediction of daily milk yield based on morning or evening milkings. Correlations are slightly lower when prediction is based on evening milkings which is in agreement with the results obtained by *Liu et al*. (2000) and our previous research (*Jovanovac et al*., 2005). Simple doubling of the morning or evening milkings, model A, gives the highest bias (±1.274 kg or 6.44% of actual daily milk yield) and highest accuracy (1.931 kg or 9.76% of actual daily milk yield). Similar results were reported in literature (*Cassandro et al.,* 1995; *Jovanovac et al*., 2005). In all models, with exception of model A, bias and accuracy were lower when daily milk yield was predicted based on morning milkings.

Table 4

Correlations between true and estimated daily milk yields, bias and accuracy of different approaches to daily milk yield prediction from morning or evening milkings

Model	Morning milking			Evening milking		
	r[1]	Bias[2]	Accuracy[3]	r[1]	Bias[2]	Accuracy[3]
A	96.48	1.274	1.931	95.34	-1.274	1.931
B	97.01	0.006	1.542	96.01	0.027	1.747
C	97.66	0.078	1.439	96.63	-0.144	1.657
D	98.16	$1.552*10^{-15}$	1.214	97.45	$4.695*10^{-17}$	1.413
E	98.18	$-2.900*10^{-15}$	1.205	97.47	$-2.130*10^{-15}$	1.409

[1]Correlations between true and estimated daily milk yields, [2]Mean difference between estimated and true yields (kg), [3]Standard deviation of the difference between estimated and true yields (kg).

Figure 1

Bias of different approaches to daily approaches to daily milk yield prediction from morning milkings

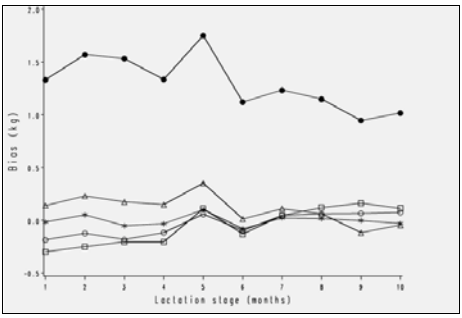
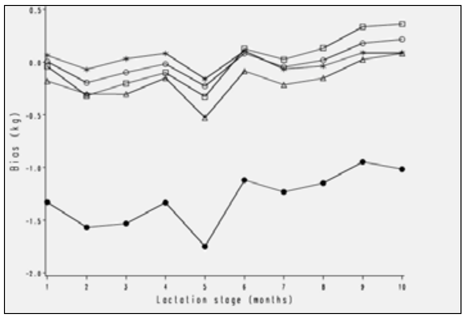


Figure 2

Bias of different milk yield prediction from evening milkings



Solid line with symbol *dot* – model A, solid line with symbol *square* – model B, solid line with symbol *triangle* – model C, solid line with symbol *circle* – model D, solid line with symbol *star* – model E.

Figure 1 and *2* show bias of different approaches to daily milk yield prediction from morning or evening milkings, respectively. Simple doubling of single yields (model A) underestimated daily milk yield if prediction is based on morning milkings and overestimated if prediction is based on evening milkings. The lowest bias for all lactation stages was observed in model E, which takes into account effect of lactation stage as lactation curve by *Ali* and *Schaeffer* (1987) which is in agreement with our previous research (*Jovanovac*, 2006).

The correlations between true and estimated daily fat contents, as well as bias and accuracy of different approaches to daily fat content prediction from morning or evening milkings are shown in *Table 5*. The most complex model, model E, gives the best fit to the data if prediction of daily fat content based on morning milkings is observed, while, if prediction of daily fat content based on evening milkings is observed, model D fits the best. Correlations are slightly lower when prediction is based on morning milkings which was expectable because correlation between evening and daily fat content was higher than the correlation between morning and daily fat content. These results are in agreement with the reported results (*Liu et al.*, 2000; *Klopčič*, 2004). With the complexity of the models, bias as well as accuracy decrease. The model A, which is model without any correction of morning or evening fat content or the most simple one, gives the highest bias and highest accuracy as well as lowest correlation between true and estimated daily fat contents. In all models, with exception of model A, bias and accuracy were lower when daily milk yield was predicted based on evening milkings.

Table 5

Correlations between true and estimated daily fat contents, bias and accuracy of different approaches to daily fat content prediction from morning or evening milkings

Model	Morning milking			Evening milking		
	r^1	Bias^2	Accuracy^3	r^1	Bias^2	Accuracy^3
A	89.29	-0.120	0.388	91.11	0.120	0.388
B	90.95	0.005	0.334	92.52	0.004	0.304
C	89.96	-0.045	0.385	91.02	0.028	0.372
D	91.67	$1.967*10^{-17}$	0.321	92.61	$-1.940*10^{-17}$	0.302
E	91.78	$-1.428*10^{-16}$	0.319	92.48	$-3.092*10^{-16}$	0.298

[1]Correlations between true and estimated daily fat contents, [2]Mean difference between estimated and true contents (%), [3]Standard deviation of the difference between estimated and true contents (%).

Figure 3

Bias of different approaches to daily fat content prediction from morning milkings

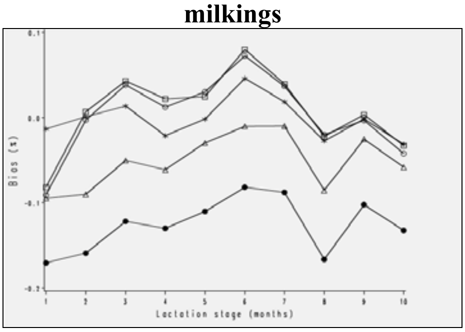
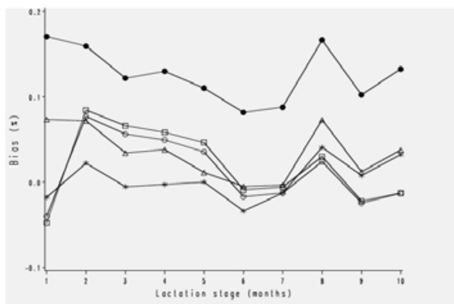


Figure 4

Bias of different approaches to daily fat content prediction from evening milkings



Solid line with symbol *dot* – model A, solid line with symbol *square* – model B, solid line with symbol *triangle* – model C, solid line with symbol *circle* – model D, solid line with symbol *star* – model E.

Figure 3 and *4* shows bias of different approaches to daily fat content prediction from morning or evening milkings, respectively. The lowest bias for all lactation stages was observed in model E if prediction is based on morning milking, while, if prediction based on evening milking is taken into consideration, model E has the lowest bias in first seven months of lactation, while at the end of lactation model D, which included evening fat content as covariate as well as effect of daily interval, shows lower bias.

The correlation between true and estimated daily protein contents increases with the complexity of the models for prediction from morning or evening milkings. That means that the most complex model, model E, gives the best fit to the data, both for prediction of daily protein content based on morning or evening milkings (*Table 6*). The differences between models are minor. Correlations are slightly higher when prediction is based on evening milkings which is in agreement with the results obtained by *Klopčič* (2004) and which differ from results reported by *Liu et al.* (2000). The model A, gives the highest bias, highest accuracy as well as lowest correlation between true and estimated daily protein contents which means that model A gives the lowest fit to the data.

Table 6

Correlations between true and estimated daily protein contents, bias and accuracy of different approaches to daily protein content prediction from morning or evening milkings

Model	Morning milking			Evening milking		
	r[1]	Bias[2]	Accuracy[3]	r[1]	Bias[2]	Accuracy[3]
A	97.24	-0.017	0.103	97.37	0.017	0.103
B	97.24	$-6.922*10^{-18}$	0.101	98.76	0.001	0.069
D	97.29	$5.415*10^{-17}$	0.100	98.78	$-1.171*10^{-16}$	0.068
E	97.48	$-3.339*10^{-16}$	0.097	98.78	$-7.790*10^{-17}$	0.068

[1]Correlations between true and estimated daily protein contents, [2]Mean difference between estimated and true contents (%), [3]Standard deviation of the difference between estimated and true contents (%).

Figure 5

Bias of different approaches to daily protein content prediction from morning milkings

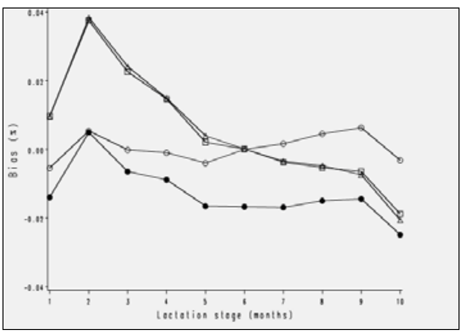
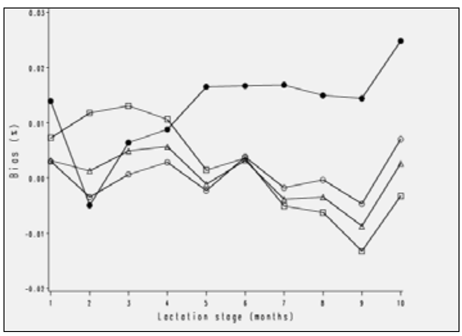


Figure 6

Bias of different approaches to daily protein content prediction from evening milkings



Solid line with symbol *dot* – model A, solid line with symbol *square* – model B, solid line with symbol *triangle* – model D, solid line with symbol *circle* – model E.

The bias of different approaches to daily protein content prediction from morning or evening milkings is shown on *Figure 5* and *6*. The lowest bias for all lactation stages, both for prediction of daily protein content based on morning or evening milkings, was observed in model E, which takes into account effect of lactation stage as lactation curve by *Ali* and *Schaeffer* (1987). This is in agreement with our previous study (*Jovanovac*, 2006).

CONCLUSIONS

Based on present study following conclusions can be made: the correlation between true and estimated daily milk yield is slightly lower when prediction is based on evening milkings, while the correlation between true and estimated daily fat as well as protein content is slightly higher when prediction is based on evening milkings. With the complexity of the models, correlation between true and estimated daily yields increases, while bias as well as accuracy decrease. Model D, which included single yields as covariate, as well as, effect of daily interval, and model E, which also included effect of lactation stage as lactation curve by *Ali* and *Schaeffer* (1987) gives the best fit to the data both for prediction of daily milk yield or milk content (fat and protein) based on morning or evening milkings. Differences between those two models were minor and statistically insignificant, so we would recommend use of model D in practice as easier to implement in routine work.

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Effect of temperature-humidity index on daily milk yield of dairy cows

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ABSTRACT

The objective of this study was to evaluate the effects of temperature-humidity index on daily milk yield of dairy cows under climate conditions in Croatia. In this study 103.569 individual test-day milk yield records of first lactation cows from Central data base of Croatian Livestock Centre were analysed. Data were collected in regular milk recording by alternative milk recording scheme from January 2005 to December 2005. According to lactation stage, cows were divided into five groups, L1 (<60 days), L2 (60–120 days), L3 (120–150 days), L4 (150–180 days), L5 (>180 days). Average temperature-humidity index, during spring, autumn and winter period was under chritical THI, so, lack of heat stress conditions characterized these periods. During the summer period, average ambient temperature and relative humidity were 22.71±4.76 °C and 70.30±9.78%, while the average THI was 70.30±7.12. Only, during July, chritical THI was exceeded. In the first 120 days of lactation, daily milk yield was higher in spring period than in the other periods, while if lactation was longer than 120 days, the highest daily milk yield production was in summer period. The highest daily fat and protein content was obtained in winter period, for all lactation stages. The linear regression of daily milk yield, fat and protein content to temperature-humidity index indicates that daily milk yield, fat and protein content slightly decreases as THI increases. Further investigations, in which effect of feeding could be eliminated, is needed.
(Keywords: temperature-humidity index, daily milk yield, dairy cows)

INTRODUCTION

Heat stress is cause of high economic losses in the dairy industry. When dairy cattle are exposed to high ambient temperatures (Ta), high relative humidity (RH) and solar radiation for extended periods, the ability of the lactating dairy cow to disperse heat decreases. Also, lactating dairy cows create a large quantity of metabolic heat. So, accumulated and producted heat joined with decreased cooling capability induced by environmental conditions, causes heat strees in the animals. Finally, heat stress induces increase of body temperature as well as decrease of feed intake and productivity. The Temperature-Humidity Index (THI) could be used to determine the influence of heat stress on productivity of dairy cows. Milk production is affected by heat stress when mean THI values are lower then 35 and higher then 72 (*Du Preez et al*., 1990). *Johnson* (1980) reported that, when THI reaches 72, milk production as well as feed intake begin to decrease. The amount of milk yield decreases during the summer period in comparision to the winter period for Holstein cows about 10% to 40% (*Du Preez et al.*, 1990). Under Mediterranean climatic conditions, milk yield drops by 0.41 kg per cow per day for each point increase in the value of THI above 69 (*Bouraoui et al.*, 2002). Beside changes in milk yield, heat stress could also cause changes in milk composition, milk somatic cell counts (SCC) and mastitis frequencies (*Rodriguez et al.*, 1985; *Du Preez et al.*, 1990). The objective of this study was to evaluate the effects of temperature-humidity index on daily milk yield of dairy cows under climate conditions in Croatia.

MATERIALS AND METHODS

In this study 103.569 individual test-day milk yield records of first lactation cows from Central data base of Croatian Livestock Centre were used. Data were collected in regular milk recording by alternative milk recording scheme from January 2005 to December 2005. At each recording, milk yields was measured in the evening or in the morning. Also, at each milking, initial time of current milking and initial time of previous milking for each animal was registrated. The interval between successive milkings was computed as the time from the beginning of previous milking to the beginning of current milking. For analysis of milk composition one sample at each milkings was taken from each cow. Daily milk yield and fat content was projected from partial values (evening or morning) according to correction factors by *DeLorenzo* and *Wiggans* (1986). Logical control of data was performed according to *ICAR* standards (2003). Variability of daily milk yield, fat and protein content is reported in *Table 1*.

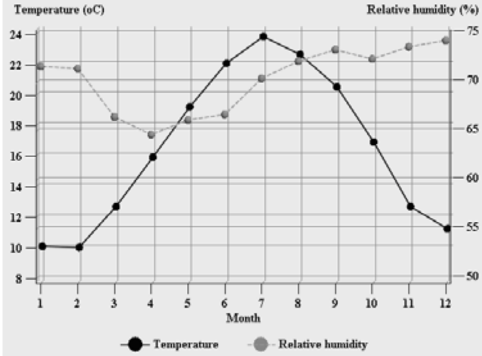
Table 1

Descriptive statistics for milk traits (n=103.569)

Trait	Mean	SD	CV	Min	Max
Daily milk yield, kg	15.87	6.33	39.90	3.00	82.32
Daily fat content, %	4.22	0.86	20.35	1.51	8.98
Daily protein content, %	3.41	0.44	13.04	1.40	6.91

Figure 1

Average measured temperature and relative humidity per months



According to lactation stage, cows were divided into five groups, L1 (<60 days), L2 (60–120 days), L3 (120–150 days), L4 (150–180 days), L5 (>180 days). Additionally, at each milking, temperature and relative humidity in stall were recorded. Daily temperature-humidity index (THI) values were calculated using the equation by *Kibler* (1964).

$$THI = 1.8 \times Ta - (1 - RH) \times (Ta - 14.3) + 32$$

Ta: measured ambient temperature in °C, RH–relative humidity as a fraction of the unit.

Distribution of average daily temperature and relative humidity according to months are shown on *Figure 1*. For estimation of the effect of season and temperature – humidity index on daily milk yield, as well as, on daily fat and protein content following fixed – effect model was used:

$$y_{ijk} = \mu + S_{ij} + b_i t_{ijk} + e_{ijk}$$

where: y_{ijk} = observation on k^{th} test-day on the j^{th} season in the i^{th} class of lactation stage,
μ= intercept,
S_{ij} = effect of season *j* nested within classes of lactation stage *i,*
b_i = regression coefficient on the temperature – humidity index nested within classes of lactation stage *i,*
t_{ijk} = temperature – humidity index on k^{th} test-day on the j^{th} season in the i^{th} class of lactation stage,
e_{ijk} = residual.

The significance of differences between the means of daily milk yield, fat and protein content within the season in relation to classes of lactation stages was tested with Scheffe test. For statistical analysis the SAS/STAT package was used (*SAS Institute Inc*., 2000).

RESULTS AND DISCUSSION

Variability of ambient temperature (Ta), relative humidity (RH) and temperatury-humidity index (THI) per season is shown in *Table 2*. *Berman* (1985) reported that the upper critical temperature for Holsteins is 25 to 26 ^{o}C, while, when THI surpass the level of 72, cows decrease milk production (*Johnson*, 1980). In the spring period, average ambient temperatute and relative humidity were 15.84±5.80 ^{o}C and 65.64±10.37%, respectively. Average THI was 59.89±8.40 and did not exceeded chritical THI (*Figure 2*). That means that envitomental conditions, in spring period, were not heat stressfull.

Table 2

Enviromental conditions during the seasons (n=103.569)

Season*	Temperature, Ta (^{o}C)			Relative humidity, RH (%)			THI		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
Spring, S1	15.84	5.80	36.66	65.64	10.37	15.81	59.89	8.40	14.03
Summer, S2	22.71	4.76	20.94	69.46	9.78	14.09	70.30	7.12	10.13
Autumn, S3	16.56	5.70	34.39	73.11	9.10	12.45	61.21	8.71	14.22
Winter, S4	10.19	3.89	38.15	72.54	9.24	12.74	51.48	5.93	11.52

*Spring, S1 (March, April and May), Summer, S2 (June, July and August), Autumn, S3 (September, October and November), Winter, S4 (December, January and February).

Figure 2

Average THI variation during the summer period

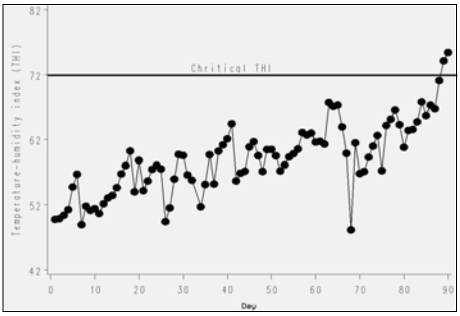
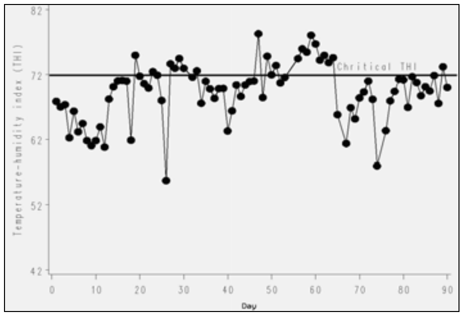


Figure 3

Average THI variation during the spring period



Average temperature-humidity index, during autumn and winter period was under chritical THI (*Figure 4* and *5*), so, lack of heat stress conditions also characterized autumn and winter period. During the summer period, average ambient temperature and relative humidity were 22.71±4.76 ^{o}C and 70.30±9.78%, while the average THI was 70.30±7.12 (*Table 2*). During July, chritical THI was exceeded (*Figure 3*).

Figure 4

Average THI variation during the winter period

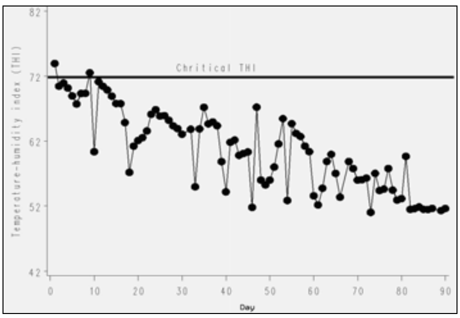
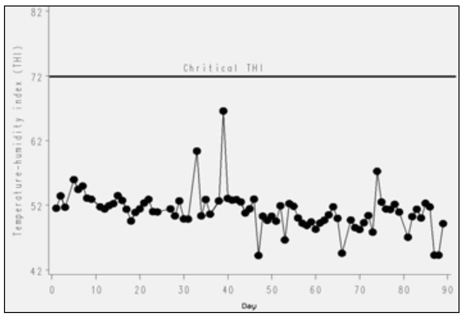


Figure 5

Average THI variation during the autumn period



Least square means of daily milk yield, fat and protein content for seasons in relation to classes of lactation stage are shown in *Table 3*. In the first 120 days of lactation (classes L1 and L2), daily milk yield production was higher in spring period than in other periods, while if lactation was longer than 120 days, the highest daily milk yield production was in summer period. The differences between seasons were statistically significant for all lactation stages.

Obtained results are not in agreement with those reported in literature (*Du Preez et al*., 1990) which could be induced by different environmental conditions in our study. Increase of the amount of milk yield during the summer period in comparision to the winter period could also be explained by inadequate and insufficient feeding during winter period. If daily fat content is taken into consideration, the differences between seasons were statistically significant and the highest content was during the winter period, for all lactation stages. Increase of daily fat content during winter period could be attributed to the increase in forage intake, while the decrease of daily fat content during the summer period in comparision to the spring period could be caused by heat stress enviroments in the summer period. The highest protein content was obtained in winter period, also, for all lactation stages. Descease of daily fat and protein content during summer period in regard to spring period was also reported by *Bouraoui et al*. (2002).

Table 3

Least square means of daily milk yield, fat and protein content for seasons in relation to classes of lactation stage

Classes of lactation stage	Season	Daily milk yield, kg	Daily fat content, %	Daily protein content, %
L1	S1	19.99^{A}	4.02^{A}	$3.09^{A, C, a}$
	S2	$18.96^{B, C, b}$	3.90^{B}	3.03^{B}
	S3	18.11^{D}	4.06^{A}	$3.12^{A, C, c}$
	S4	$18.41^{B, C, c}$	4.23^{C}	3.17^{D}
L2	S1	19.03^{A}	3.91^{A}	3.18^{A}
	S2	18.77^{A}	3.78^{B}	3.09^{B}
	S3	16.62^{B}	3.95^{A}	3.21^{C}
	S4	16.50^{B}	4.13^{C}	3.22^{C}
L3	S1	17.46^{A}	$4.01^{A, C, a}$	3.34^{A}
	S2	17.81^{A}	3.90^{B}	3.24^{B}
	S3	15.45^{B}	$4.08^{A, C, c}$	$3.37^{A, C}$
	S4	14.70^{C}	4.22^{D}	3.40^{C}
L4	S1	16.20^{A}	$4.10^{A, C, a}$	3.41^{A}
	S2	17.10^{B}	3.98^{B}	3.32^{B}
	S3	14.91^{C}	$4.16^{A, C, c}$	3.45^{C}
	S4	13.31^{D}	4.34^{D}	3.48^{C}
L5	S1	13.11^{A}	4.41^{A}	3.63^{A}
	S2	14.59^{B}	4.23^{B}	3.51^{B}
	S3	13.59^{C}	4.45^{C}	3.68^{C}
	S4	12.22^{D}	4.59^{D}	3.71^{D}

*The values, within classes of lactation stages, marked with the same letter are not (highly) significantly different (P>(0.01) 0.05).

Figure 6 shows relation of daily milk yield to temperature-humidity index according to classes of lactation stage. The negative slope of the regression line, for all lactation stages with exception of stage L4, indicates that milk production decreases as THI increases. The most intensive decrease is in second lactation stage or between 60th and 120th day of lactation (*Table 4*). Higher decrease of daily milk yield in relation to temperature-humidity index in mid-lactating Friesian–Holstein cows (144 to 150 days postpartum) was reported by *Bouraoui et al*. (2002).

Decrease of daily fat and protein content in regard to temperature-humidity index according to classes of lactation stage is shown on *Figure 7* and *8*.

Figure 6

Relation of daily milk yield to temperature-humidity index

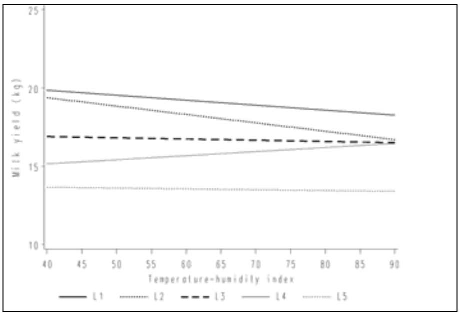


Figure 7

Relation of daily fat content to temperature-humidity index

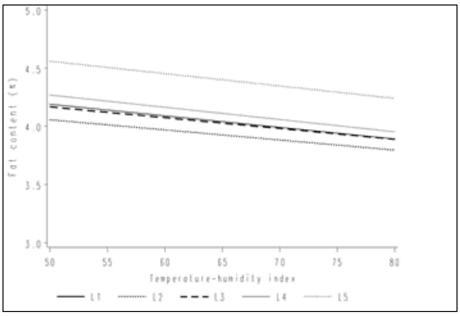
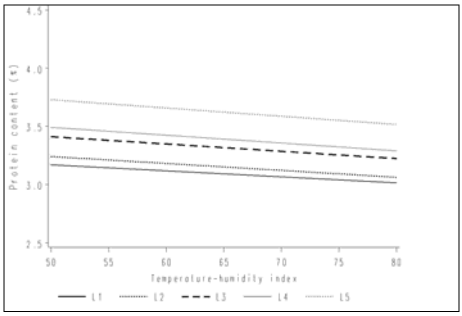


Figure 8

Relation of daily protein content to temperature-humidity index



The negative regression coefficient (*Table 4*), for all lactation stages, indicates that daily fat and protein content slightly decreases as THI increases.

Table 4

Regression coefficient on the temperature – humidity index in relation to classes of lactation stage

Trait	Classes of lactation stage				
	L1	L2	L3	L4	L5
Daily milk yield, kg	-0.0317	-0.0535	-0.0077	+0.0259	-0.0053
Daily fat content, %	-0.0100	-0.0088	-0.0093	-0.0106	-0.0106
Daily protein content, %	-0.0052	-0.0059	-0.0063	-0.0067	-0.0071

CONCLUSIONS

Based on present research it could be concluded that in the first 120 days of lactation, daily milk yield was higher in spring period than in the other periods, while if lactation was longer than 120 days, the highest daily milk yield production was in summer period. The highest daily fat and protein content was obtained in winter period, for all lactation stages. The linear regression of daily milk yield, fat and protein content to temperature-humidity index indicates that daily milk yield, fat and protein content slightly decreases as THI increases. Further investigations, in which effect of feeding could be eliminated, is needed.

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Effect of hot season and type of floor on the microclimate conditions in the pens of beef cattle intensive farms

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ABSTRACT

A study was conducted on a sample of 20 beef cattle farms to verify the effect of season and type of floor on microclimate parameters. Ten farms had multiple pens with slatted floor and 10 with littered floor. Temperature, humidity, THI, NH_3 and CO_2 were measured with specific instruments during two inspections in summer and winter time. A short interview was also made with the stockman about the adoption of several managing strategies to limit heat stress on animals during the hot period. As expected temperature and THI values recorded in the summer were significantly higher than the winter ones while there were no difference due to the type of floor in the pen. The THI values observed in littered and slatted floor pens (78.6 and 79.0 respectively) in the summer inspection were over 75, the recognized threshold for heat stress in cattle. Noxious gasses concentrations were unaffected by season and type of floor resulting far below their toxic concentration for cattle. According to the stockman interviews, there is a clear perception of the detrimental effect of the hot season on cattle welfare which impairs feed intake. Some preventive strategies are applied in most cases but they appear insufficient and often not operative when needed.
(Keywords: beef cattle, microclimate conditions, hot season, type of floor)

INTRODUCTION

Farm microclimate conditions are a factor that can significantly affect beef cattle performance (*Mitlöhner et al*., 2001). Critical summer weather conditions have shown to impair the animal welfare by increasing body temperature and reducing feed intake (*Lefcourt* and *Adams*, 1996). Under intensive rearing systems, the effect of the hot climate on the beef cattle response can be exacerbated by the heat increment induced by feeding diets rich in concentrates (*Mader*, 2003). Moreover, the health status of the animal can be worsened by the enhanced concentrations of noxious gasses promoted by an increased fermentation of bedding material and animal wastes. Reference thresholds for temperature, humidity and noxious gasses concentrations can be found in the literature in order to assess the welfare of confined beef cattle during the summer (*SCAHAW*, 1999 and 2001; *Holt et al*., 2004). The aim of present study was to evaluate the change of some of these microclimate parameters from winter to summer time in a sample of Italian beef cattle farms located in the Po Valley which adopted a different type of floor in their pens. The farmers attitude towards specific management strategies to be applied in the attempt to relieve cattle heat stress was also recorded by a focused interview.

MATERIALS AND METHODS

The study was conducted on a selected sample of 20 intensive beef cattle farms located in the Veneto Region (Italy) within a maximum distance of 60 km at iso-climatic weather conditions. All farms had close barns, ten of them had multiple pens with straw bedded floor while the remaining 10 had slatted floor pens. All farms were visited in two times of the year, August 2005 and February 2006, in order to compare the microclimate conditions during the warm and the cold seasons. The inspection was carried out between 11.00 AM and 02.00 PM by a trained technician who was in charge to measure several microclimate parameters. Temperature and humidity were recorded by a HD206-2 Datalogger (Delta Ohm, Padova, Italy) while the concentrations of CO_2 and NH_3 were detected by a Drager CMS (Dräger Safety AG & Co. KGaA, Luebeck, Germany). All the measurements were taken holding the instruments inside the pen at 150 cm of height in order to closely resemble to breathing height of the animal in standing position. The ventilation systems were never working at the time of data recording and this situation was not imposed by the technician. Experimental temperature and humidity data were used to calculate the Temperature Humidity Index (THI) according to the following equation: THI=TF-(0.55-[0.55*RH/100)]*(TF-58) (*Ominski et al*., 2002) where T F is the temperature in Fahrenheit degrees and RH is the relative humidity value.

At the end of the summer visit, a short interview was also made to each farmer in order to know possible changes in the management of the animals and in their behaviour during the hot season. Most of the questions were about the adoption of devices and strategies capable to alleviate heat stress of cattle. A few questions were also addressed to assess change in cattle feed intake and health problems during the hot season.

All microclimate data were submitted to a repeated measures analysis of variance within GLM procedure of *SAS System* (1990). Season, pen type of floor and their interaction were the factors included in the statistical model. The minimum threshold for the statistical significance was $P<0.05$.

RESULTS AND DISCUSSION

The general description of the selected sample of farms used in the study is reported in *Table 1*. The farm size, measured as number of cattle, was similar for both types of floor either as average or as standard deviation. Animals on slats were mainly of French origin while dual purpose breeds cattle number was higher in the farms with littered floor.

Table 1

General description of the sample of beef cattle farms used in the study

Number of farms	**Type of floor**	
	Litter	**Slats**
	10	10
Cattle, (mean ± SD)	592.0 ± 570	575 ± 557
Main cattle genotypes: (n. of farms)		
French pure and crossbred	6	9
Double purpose breeds	4	1
Space allowance, (m^2/head)	5.0 ± 1.9	3.1 ± 0.4

Microclimate conditions

Average temperatures recorded in the two periods of the year are shown in *Table 2*. As expected, summer values were significantly higher than the winter ones whereas no difference was observed for the different type of floor in the pen.

Humidity mean values were not affected by season and type of floor (*Table 2*). However, when considering the effect of these parameters on the animal welfare, it is more realistic to combine them in a single index like the THI (*NOAA*, 1976). The calculation of THI in the present study showed a significant difference of the season without any effect due to the type of floor (*Table 2*). The values measured in the hot period resulted above 75 which is recognized as the minimum threshold of heat stress for cattle (*Mader* and *Davis*, 2004; *Holt et al*., 2004; *West*, 2003).

The average concentrations of two noxious gasses like NH_3 and CO_2 are reported in *Table 2*. The season, the type of floor and their factorial combination had no effect on these parameters.

Table 2

Effect of season and type of floor on microclimate parameters in the pen

		Summer		Winter		Significance			rmse
		litter	slats	litter	slats	season	floor	season*floor	
Temperature	°C	29.2	29.3	6.8	7.5	P<0.0001	ns	ns	3.90
Humidity		59.6	60.7	63.3	54.3	ns	ns	ns	13.06
THI		78.6	79.0	47.2	48.5	P<0.0001	ns	ns	5.66
NH_3	ppm	3.2	5.8	3.0	0.9	ns	ns	ns	5.77
CO_2	ppm	970.0	829.0	776.0	799.0	ns	ns	ns	431.15

The values observed for both gasses in the summer inspection tended to be higher than those recorded in winter but their difference did not reach the minimum threshold for the statistical significance due to the great variation observed within factor. In the case of NH_3 the P value for the season contrast resulted <0.07. According to *SCAHAW* (2001), the welfare of beef cattle is impaired when the levels of NH_3 and CO_2 are higher than 20 ppm and 5000 ppm respectively. The comparison of these values with those recorded in the present study, even during hot season, showed the absolute lack of risk for the animals.

Farmers interview

The frequency of the answer of the farmers as regards to the adoption of a set of solutions to alleviate cattle heat stress during the summer are reported in *Table 3*.

Most of the farms and those adopting the litter in particular way had ventilation systems to be used in hot days. Artificial ventilation is considered a good solution to alleviate the stress of animals that have to cope with hot climate conditions (*SCAHAW*, 1999) and the THI values recorded during our summer inspection (*Table 2*) should have strongly advised to their adoption. However, it must be pointed out that none of them were operating during the inspection.

The strategy to shift the time of feed distribution towards the late evening hours is considered a way to stimulate feed intake in cattle under heat stress conditions (*Mader*, 2002; *Holt et al*., 2004). This guideline was adopted by about half of the farms of our sample but, according to stockman report it was not able to prevent the drop of intake by the animals in the summer (*Table 4*). The increase in the watering system is also a recommended strategy to compensate the greater water losses due to the activation of thermoregulatory mechanisms by the animals (*Shalit et al*., 1991). However none of the farms provided additional waterers in the hot season (*Table 3*) and this decision could also explain the reduced feed intake by cattle. An adaptation behaviour of cattle under hot critical conditions is to increase the distance between animals (*SCAHAW*, 1999) and an increase in the space allowance in the pen should promote this behaviour. This strategy seems to be adopted particularly by farms with slatted floor pens (*Table 3*) where cattle are housed at a higher density (*Table 1*).

Table 3

Frequency of adoption of different solution to alleviate heat stress during the summer in beef cattle farm with different type of floor in the pens

	litter		**slats**		**total**
Management strategies:	Yes	No	Yes	No	
Ventilation systems	8	2	6	4	20
Change in time of diet distribution	5	5	4	6	20
Additional waterers in the pen	0	10	0	10	20
More frequent litter renewal	6	4	-	-	10
Increase in space allowance	4	6	6	4	20

Table 4

Effects of the summer microclimate conditions on beef cattle intake and health status as reported by the stockman of farms with different types of floor in the pens

Effects	**litter**		**slats**		**total**
	Yes	No	Yes	No	
Decrease in animal feed intake	8	2	6	4	20
Increase in pathologies	5	5	5	5	20
Increase in lameness	2	8	4	6	20

When the stockman was questioned about the incidence of cattle health problems in the summer in comparison to the cooler seasons, there was not a clear indication for an increase in pathologies (respiratory problems, tail necrosis etc) in both types of floor (*Table 4*). On the contrary, the number of lameness animals seems not to be increased in the hot season particularly in pens with littered floor (*Table 4*). A possible behavioural explanation for this result might be the reduced motivation of the animals to stand and move when exposed to hot temperature. *Mitlöhner et al.* (2001) observed a significant increase in lying behaviour in beef heifers exposed to heat stress without any shading and cooling device.

CONCLUSIONS

The microclimate conditions in the rearing facilities represent an outstanding issue in the assessment of animal welfare. The present study carried out on a sample of beef farms located in the main area for beef production of Italy, the Eastern Po Valley, has shown that, regardless of the type of floor, cattle are likely to be exposed to heat stress during the summer season. Farmers are aware of this problem which results in a lower feed intake by the animals but their preventive strategies are limited and often not operative when needed. The concentrations of two noxious gasses like NH_3 and CO_2 measured in the pens during the summer inspection did not differ from the values recorded in winter time and no type of floor effect on these parameters was observed. The experimental data for both gasses were far below their toxic concentration for cattle suggesting that they are a minor source of risk for the welfare of the animals in the housing structures for beef cattle of the Po Valley.

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Different parameters affecting body weights of Charolais and Limousine calves from birth to weaning

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ABSTRACT

Birth weight and body weight at the beginning, in the middle and at the end of grazing season of Charolais (312) and Limousine (167) calves at the Educational and Research Animal Husbandry Centre Logatec(Slovenia) born from 1995 to 2005 were analysed. Fixed effect of breed, sex, parity, year, age of calves and random animal effect were included. Breed, sex and year influenced birth weight and body weight at the beginning, in the middle and at the end of grazing season. Parity influenced only birth weight and body weight at the beginning of grazing season. Age of calves influenced body weight at the beginning and in the middle of grazing season, but this effect was not shown at the end of grazing season. Variance and covariance components for direct additive genetic effect were estimated by REML method in the VCE5 package. Estimated heritability for birth weight, body weight at the beginning, in the middle and at the end of grazing season were 0.62; 0.25; 0.25 and 0.26, respectively. Genetic correlation with birth weight for body weight at the beginning was 0.78, for body weight in the middle was 0.51 and for body weight at the end of grazing season was 0.40.
(Keywords: Charolais, Limousine, calves, body weight, heritability)

INTRODUCTION

In Slovenia suckler cows represent about 40% of total cow population and the percent is still increasing. The most important reason for widening suckler herds is the introduction of milk quotas because of rapidly increasing milk production. Milk production per cow has always been very intensive and is still rising. Consequently, the number of dairy cows has reduced. It can be expected that less and less calves from milk herds will be suitable for beef production.

Besides providing quality beef, rearing of suckler cows has numerous macro-economical advantages in comparison to dairy production, namely milk market relief, rearing in marginal regions, preservation of population and cultural landscape and keeping the agricultural lands in function. From the point of view of sustainable agricultural production such rearing is really beneficial (*Čepon* and *Žgur*, 2001).

The most important product for sale from suckler herd is a weaned calf. High percent of weaned calves in herds, about 90–95%, is recommended. Culled cows represent just a small part of income. Weaned calves are very suitable for slaughter or for fattening. Consumers require ecological production of quality beef from cow-calf systems.

The aim of this study was to determine the environmental and genetic parameters influencing weaning weight (weight at the end of grazing season) of Charolais and Limousine calves from a suckler herd. Birth weight, weight at the beginning and weight in the middle of grazing season were also analysed.

MATERIALS AND METHODS

Our research included 312 Charolais (CHA) and 167 Limousine (LIM) calves reared on Educational and Research Animal Husbandry Centre Logatec (Slovenia). CHA calves were born in years 1995–2005, while LIM calves between 1996 and 2005. Cows in the herd calved in late winter or spring (January – June). The average grazing season (149.3 days) lasted from the beginning of May to the end of October. The end of grazing season coincided the weaning period/time. Weaned calves were on average 196.4 days old (6.5 months). In grazing season cows and calves had no additional concentrate, except mineral–vitamin mixture fed *ad libitum*.

Pedigree data included calves sires, dams and grandparents. Grand sires were known in 90% on a male side and in 100% on dam side. In the analysed period, 37 sires had offspring in the herd. This included sires for natural service as well as artificial insemination (AI). On average, sires of natural service had more offspring in herd than sires of AI.

Data on birth weight, weight at the beginning, in the middle and at the end of grazing season were analysed. Calves were weighed at the beginning (60.8 days of age), in the middle at the average age 135.9 days and at the end of grazing season at the average age of 196.4 days (*Table 1*).

Table 1

Descriptive statistics for body weights till weaning

Breed	Sex		BW (kg)	WB (kg)	WM (kg)	WE (kg)
Charolais	Male	n	160	116	115	125
		$\bar{x}$ ± SD	48.1 ± 6.6	100.1 ± 23.9	202.7 ± 50.5	270.6 ± 50.8
	Female	n	152	109	111	127
		$\bar{x}$ ± SD	45.9 ± 6.1	97.6 ± 23.0	194.5 ± 47.7	252.2 ± 52.5
Limousine	Male	n	71	37	62	61
		$\bar{x}$ ± SD	43.5 ± 4.4	89.2 ± 22.7	162.1 ± 51.1	214.0 ± 57.9
	Female	n	96	46	75	74
		$\bar{x}$ ± SD	41.0 ± 4.3	88.8 ± 21.4	150.3 ± 47.4	202.8 ± 46.5

BW: birth weight, WB: body weight at the beginning of grazing season, WM: body weight in the middle of grazing season, WE: body weight at the end of grazing season, n: number of calves, $\bar{x}$:mean, SD: standard deviation.

Based on preliminary analyses, different models were used for body weights analyses of calves. Interactions between different fixed effects were not significant. Main effects only were used for further analyses. The effects of breed, sex, parity and year as fixed effects were included in all models ([1], [2], [3], [4]).

Age of calves at the beginning, middle and the end of grazing season were included as linear regression in the models [2], [3], [4] for corresponding weights. Additionally, the age of calves at the beginning of grazing season was fitted in the models for weight in the middle and at the end of grazing season, since from previous analyses on our populations (*Čepon*, 1990; *Čepon* and *Žgur*, 2001) its effect was expected. Random animal effect was also included in all models ([1], [2], [3], [4]).

Fixed part of the model was analysed by GLM procedure of statistical package SAS/STAT (*SAS* Institute Inc., 2001). The general least square method was used for this. Variance and covariance components for direct additive genetic (animal) effect were estimated by REML method in the VCE5 package (*Kovač et al*, 2002). The model [1] was used for the estimation of birth weight, the model [2] for the estimation of weight at the beginning, the model [3] for the weight in the middle and the model [4] for the weight at the end of grazing season in the multiple trait analysis.

$$y_{ijkl} = \mu + B_i + S_j + C_k + Y_l + a_{ijkl} + e_{ijkl} \quad [1]$$

$$y_{ijklm} = \mu + B_i + S_j + C_k + Y_l + b_I(x_{ijklm} - \bar{x}) + a_{ijklm} + e_{ijklm} \quad [2]$$

$$y_{ijklm} = \mu + B_i + S_j + C_k + Y_l + b_I(x_{ijklm} - \bar{x}) + b_{II}(w_{ijklm} - \bar{w}) + a_{ijklm} + e_{ijklm} \quad [3]$$

$$y_{ijklm} = \mu + B_i + S_j + C_k + Y_l + b_I(x_{ijklm} - \bar{x}) + b_{II}(z_{ijklm} - \bar{z}) + a_{ijklm} + e_{ijklm} \quad [4]$$

Where:
y_{ijklm} = body weights (BW, WB, WM, WE) kg;
B_i = breed; i = 1, 2;
S_j = sex; j = 1, 2;
C_k = parity; k = 1, …, 7;
Y_l = year; l = 1, … 11;
x_{ijklm} = age of calves at the beginning of grazing season, days;
w_{ijklm} = age of calves in the middle of grazing season, days;
z_{ijklm} = age of calves at the end of grazing season, days;
a_{ijklm} = direct additive genetic effect;
e_{ijklm} = residual.

RESULTS AND DISCUSSION

Analysis of variance for birth weight, weight at the beginning, in the middle and at the end of grazing season for the fixed part of models is shown in *Table 2*. Proportion of variability explained by Model 1 was 32%. Birth weight of Charolais and Limousine calves depended on the breed ($p<0.001$), sex ($p<0.001$), parity ($p=0.036$) and year ($p<0.001$). The difference in birth weight between Charolais (LSM=46.9 kg) and Limousine (LSM=41.3 kg) calves was 5.6 kg. Higher difference (7.6 kg) between BW of CHA (47.1 kg) and LIM (39.5 kg) calves in France was reported by *Phocas* and *Laloë* (2004). Similar results of the effect of year ($p<0.001$), sex ($p=0.002$), breed ($p<0.001$) and parity ($p=0.019$) were shown by *Čepon* and *Žgur* (2001) in previous analyses for calves born from 1996 to 2000 in the same herd. *Krupa et al*. (2005) also found that sex ($p<0.001$) and breed ($p<0,001$) affected birth weight of calves of various beef breeds in a field test (cow–calf grazing system) in Slovakia.

Model 2 was used to explain 59% of variability for weight at the beginning of grazing season, when calves were 60.8 days old. All included parameters (breed, sex, parity, year, age at the beginning of grazing season) affected weight at the beginning of grazing season. Charolais calves (LSM=97.9 kg) were 5.8 % heavier than Limousine (LSM=92.2 kg). Males (LSM=97.4 kg) were 4.7 kg heavier than females (LSM=92.7 kg).

Weighing in the middle of grazing season was performed at 135.9 days on average. Coefficient of determination in Model [3] was the highest (67%). Among all included parameters parity did not affect on WM (p=0.153). Charolais calves weighted 203.4 kg (LSM) and Limousine 186.5 kg (LSM). *Krupa et al*. (2005) showed the effect of breed ($p<0.001$) and sex ($p<0.001$) on weight at 120 days at six beef breeds.

Variability explained for weight at the end of grazing season was 60%. The WE (196.4 days on average) corresponded weaning weight and depended on breed ($p<0.001$), sex ($p<0.001$) and year ($p<0.001$). The difference in WE between Charolais (LSM=266.8 kg) and Limousine (LSM=240.2 kg) calves was 26.6 kg. Males (LSM=263.7 kg) were 7.7% heavier than females (LSM=243.3 kg). *Makulska et al*. (2003) reported the following weight at 210 days of Charolais (253 kg) and Limousine (245 kg) calves in Poland. *Phocas* and *Laloë* (2004) found out weaning weight for CHA (279.8 kg) and for LIM (258.3 kg) from herds in France. *Goyache et al.* (2003) showed similar effect of sex ($p<0.001$) on weaning weight at the local Spanish beef breed called Asturiana de los Valles. *Krupa et al*. (2005) also reported the effects of breed ($p<0.001$) and sex ($p<0.001$) on weaning weight of beef calves. The age of calves at the beginning of grazing season did not affect the weights in the middle and at the end of grazing season, which was in the opposite of results from *Čepon* (1990) and *Čepon* and *Žgur* (2001).

Table 2

Analysis of variance for birth weight and body weight at the beginning, in the middle and the end of grazing season by GLM

Source of variability	d.f.	p – value			
		BW	WB	WM	WE
Breed	1	<0.001	0.009	<0.001	<0.001
Sex	1	<0.001	0.011	<0.001	<0.001
Parity	6	0.036	0.050	0.153	0.469
Year	10	<0.001	<0.001	<0.001	<0.001
Age (beginning)	1	-	<0.001	0.373	0.298
Age (middle)	1	-	-	0.038	-
Age (end)	1	-	-	-	0.558
R^2		0.32	0.59	0.67	0.60

BW: birth weight, WB: body weight at the beginning of grazing season, WM: body weight in the middle of grazing season, WE: body weight at the end of grazing season, d.f.: degrees of freedom, R^2: coefficient of determination.

Genetic and phenotypic variance of BW, WB, WM and WE are presented in *Table 3*. Phenotypic variance of BW (30.72 kg^2) is higher than those found by *Phocas* and *Laloë* (2004) for BW at CHA (20.0 kg^2) and at LIM (7.5 kg^2) calves. The same authors also reported about variance for weaning weight in CHA (1141 kg^2) and in LIM (662 kg^2) weaned calves which are very similar to our result (1102.33 kg^2). Genetic and phenotypic standard deviations were computed for easier interpretation and comparison with literature. Genetic standard deviation was 4.35 kg, 7.67 kg, 13.28 kg and 17.11 kg for BW, WB, WM and WE, respectively. Lower genetic standard deviation for BW had Charolais (2.45 kg) and Limousine (1.70 kg) calves reared in Czech Republic (*Jakubec et al*., 2003), who also had lower genetic SD for weight at 210 days (LIM; SD=14.51 kg) and similar SD to our result (CHA; SD=16.53 kg).

Table 3

Genetic variance, phenotypic variance, genetic covariance (above diagonal) and phenotypic covariance (below diagonal) between BW, WB, WM and WE

	σ^2_g (kg^2)	σ^2_{ph} (kg^2)	BW	WB	WM	WE
BW	18.97	30.72		26.13	29.56	29.55
WB	58.89	232.89	43.75		49.28	66.97
WM	176.13	700.69	46.76	266.78		209.59
WE	292.68	1102.33	49.71	268.68	705.81	

σ^2_g : genetic variance, σ^2_{ph} : phenotypic variance, BW: birth weight, WB: body weight at the beginning of grazing season, WM: body weight in the middle of grazing season, WE: body weight at the end of grazing season.

Phenotypic standard deviation was 5.54 kg, 15.26 kg, 26.47 kg and 33.20 kg for BW, WB, WM and WE. *Jakubec et al*. (2003) also reported similar phenotypic SD for BW at CHA (4.90 kg) and LIM (3.39 kg). Standard deviation for WE at CHA (33.05 kg) in LIM (29.01 kg) was also similar to our results. Phenotypic (above diagonal) and genetic (below diagonal) covariance are also shown in *Table 3*.

Heritability estimated in this study for BW, WB, WM, WE were 0.62; 0.25; 0.25; 0.26 and are shown on diagonal in *Table 4.* The highest values of heritability coefficient were estimated for BW (0.62). Heritabilities for WB, WM and WE were very similar. *Phocas* and *Laloë* (2004) estimated lower heritability for BW at CHA (h^2=0.33) and LIM (h^2=0.38), but *Crews et al*. (2004) estimated higher heritability for Canadian CHA (h^2=0.53). Differences among our results and literature exist because only one herd with very equal environment was included. Heritability of the similar traits for Angus (*Anonymous*, 2005a) was 0.38 for birth weight and 0.18 for the weight at 200 days. *Phocas* and *Laloë* (2004) estimated also heritability for weaning weight which was 0.13 (CHA) and 0.29 (LIM) and was similar to our results (h^2=0.26). Heritability for weight at 205 days at Canadian CHA was 0.22 (*Crews et al*., 2004). Genetic correlations are presented above diagonal in *Table 4*. Genetic correlation between BW and WE was 0.40. In literature, 0.39 was reported for CHA and 0.44 for LIM (*Phocas* and *Laloë* (2004), and 0.66 for Angus cattle (*Anonymous*, 2005b). Between the WB, WM, WE, high genetic correlations were estimated.

Table 4

Heritability (on the diagonal) for birth weight, weight at the beginning, in the middle and the end of grazing season, genetics correlations (above diagonal) among them with standard errors

	BW	WB	WM	WE
BW	0.62 ± 0.07	0.78 ± 0.11	0.51 ± 0.14	0.40 ± 0.13
WB		0.25 ± 0.08	0.48 ± 0.19	0.51 ± 0.17
WM			0.25 ± 0.08	0.92 ± 0.07
WE				0.26 ± 0.07

BW: birth weight, WB: body weight at the beginning of grazing season, WM: body weight in the middle of grazing season, WE: body weight at the end of grazing season.

CONCLUSIONS

Birth weight, weight at the beginning, in the middle and the end of grazing season of Charolais and Limousine calves at the Educational and Research Animal Husbandry Centre Logatec were analysed. Fixed effect of breed, sex, parity, year, age of calves and random animal effect were included. Breed, sex and year influenced birth weight and weight at the beginning, in the middle and at the end of grazing season. Parity influenced only birth weight and weight at the beginning of grazing season. Age of calves at the beginning of grazing season influenced weight at the beginning, age of calves in the middle of grazing season influenced body weight in the middle of grazing season.

Genetic and phenotypic variance and covariance components for random animal effect were estimated and were in the same range as estimated (co)variance components from the literature. Estimated heritability for birth weight, weight at the beginning, in the middle and at the end of grazing season were 0.62; 0.25; 0.25 and 0.26, respectively. Genetic correlation with birth weight for weight at the beginning was 0.78, for weight in the middle was 0.51 and for weight at the end of grazing season was 0.40.

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Carcass traits of young Simmental bulls and heifers classified according to the EUROP system

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ABSTRACT

The objective of this investigation was to determine the carcass traits (weight and measurements, dressing percentage, cooling loss, shares of separated fat and dissected muscle, fat, bone and tendon tissues as well as shares of beef cuts of different retail categories) of young Simmental bulls (n=13) and heifers (n=13) classified according to EUROP system which were produced as Croatian baby beef destined for Italian market. The classification showed a favorable conformation of both, bulls and heifers with about one third carcasses graded as highest E class. The heifers fatness was less favorable and almost half of carcasses were classified as high fat class (4) and thus less valuable. The heifers over fatness was confirmed by significantly higher amount of trimmed carcass fat and higher share of fat tissue and lower share of muscle than bulls after carcass dissection. The carcasses of both sex classified as most valuable E class had the lowest proportion of muscle which imply a need for a additional improvement of conformation assessment practice. The differences between bulls and heifers in dressing percentage, carcass cooling loss, shares of Milanese cut, bone and tendon tissue as well as shares of cuts of different beef retail categories in the carcass were relatively small.
(Keywords: Simmental cattle, baby-beef, carcass traits, EUROP system)

INTRODUCTION

Croatia has traditionally been the exporter of livestock and beef, with Italy as the most important export destination (*Pankretić*, 1998). The most exported product is the ¨baby beef˙ – meat from carcasses of corn-fattened Simmental cattle at the age of about 12 months. It is generally cut and marketed in the form of ¨Milanese cut˙, consisting of the most valuable carcass parts. Croatian baby beef sells mostly in the region of Toscana where it is very appreciated among consumers and usually used for the preparation of the famous Florentine steak (*Kolega et al.*, 2003). The market value of a beef carcass is principally determined by weight, conformation and proportions of lean and fat. Since 2004, the EUROP classification system for beef carcasses evaluation has been introduced in the slaughter plants in Croatia (NN 20/2004). This system determines the carcass conformation (meat deposition) and adiposity (fatness) class by common grading scheme facilitating reasonable financial settlement with the producer and carcass trade on European Union market for comparable prices (*Kallwet* and *Henning*, 1998; *Florek* and *Litwinezuk*, 2002; *Wajda* and *Daszkiewicz*, 2002).

The objective of this investigation was to determine the carcass traits of young Simmental bulls and heifers classified according to EUROP system which were produced as Croatian baby beef destined for Italian market.

MATERIALS AND METHODS

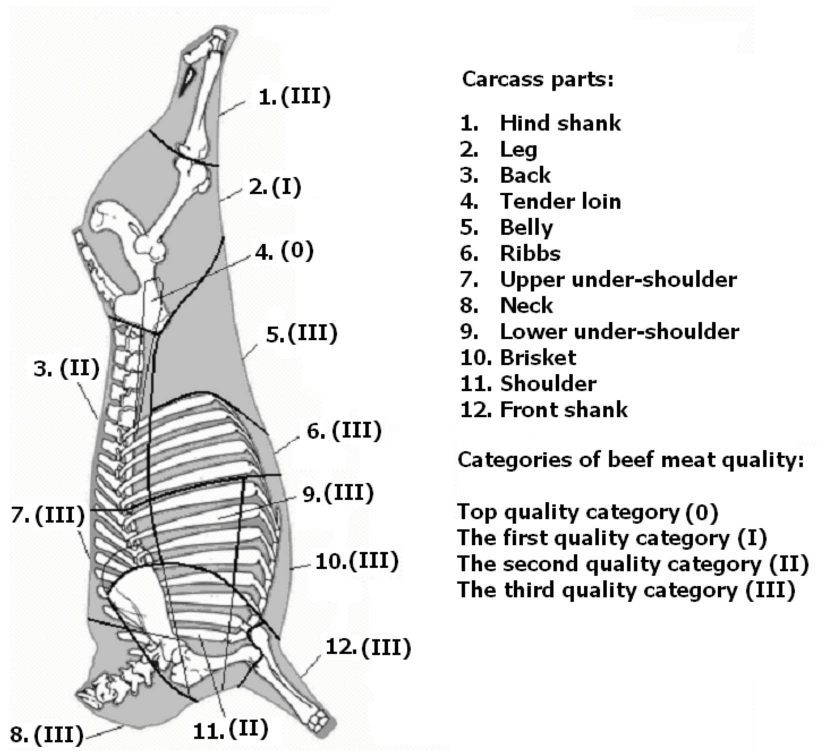
The investigation was conducted on twenty six Simmental cattle (a total of 13 bulls and 13 heifers). The animals were calved over the period Oct.-Dec. 2004 on the family farms in the northwestern part of Croatia and bought at the beginning of May 2005 by¨Baby Beef Breeders Association¨, Gudovec for fattening purposes. The fattening took place at the same farm in the two nearby fattening units under the similar conditions for all animals. The mean weight of bulls at the start of fattening was 294 kg, while that of heifers was 288 kg. They were fed corn grain silage *ad libitum*, complemented with approximately 1 kg of concentrate and 1 kg of hay per animal daily for about 5 months. The mean weight of bulls before slaughter reached 510 kg with average daily gain of 1.4 kg, while that of heifers was 455 kg with 1.1 kg of average daily gain. At the time of slaughter the animals were at the age of about 12 months. The slaughter was carried out in five batches during 6 weeks (September – November, 2005) in the Meat Industry ¨*IMI*¨, Ivanec. This abattoir is approved for export to European Union (Export number: 214, registered 05.05.2004). The animals were transported and slaughtered according to established regulations (NN 20/04, NN 116/05). Hot carcass weight (HCW) was measured without removing the subcutaneous fat and maintaining the kidney and pelvic fat. The tail was removed. Dressing percentage (DP) was calculated with formulae: (hot carcass weight / live weight before slaughter) x 100. The excessive covering fat on round and groin area and internal fat depots (kidney and pelvic fat) from right side were trimmed and weighed together to obtain the average value of separated fat (SFAT). Once the dressing was finished, the classification according to the EUROP system was performed on hot carcasses by authorized classifier (Agroinspekt d.o.o.). The classification included the determination of carcass conformation (CONF, expressed as E-excellent, U-very good, R-good, O-fair or P-poor) and carcass fatness (FAT, fat cover expressed as 1-very low fat, 2-low fat, 3-average fat, 4-high fat or 5-very high fat). Several carcass measurements were taken on the right half by meter: carcass length (CL, measured from the anterior edge of symphysis pubis to the anterior edge of the first rib), length of hind leg (LL, measured from the middle of knee joint in the straight line to the anterior edge of the symphysis pubis) or by tape: perimeter of leg (PL, measured as maximum horizontal contour of a leg at the symphysis pubis level). After cooling for 48 hours at 4 °C, the carcasses were weighed once more to determine the cold carcass weight (CCW). Carcass cooling loss (CCL) was calculated with formulae: (HCW-CCW)/HCWx100. The carcass tissue composition was assessed by full dissection of right half of each carcass. The halves were first divided into the quarters by cut between eighth and ninth rib and then into the parts according to scheme in *Figure 1* (DLG method, *Scheper* and *Scholz*, 1985). Each joint was weighed and dissected into the muscle (M), bone (B), fat (F) and tendon (T). The total weight of separated tissues was used as the denominator for calculating proportions of particular tissue in the carcass. The evaluation of ¨Milanese cut¨ (MC, as % of HCW) included hind shank, leg, back and tender loin. Finally, the proportions of beef cuts of different retail categories (*Figure 1*) in the carcass were evaluated. The data were analyzed by analysis of variance using the GLM procedure (*SAS*, 1999).

RESULTS AND DISCUSSION

The carcass traits of both sex categories within the EUROP conformation and fatness classes are presented in *Table 1* and *Table 2*. As regards conformation (*Table 1*), the distribution of the carcasses of bulls and heifers within classes were equally favorable. More than a half of carcasses (7 or 54% in each category) were classified as class U, 30.8% carcasses (4 in each category) were classified as class E and the two carcass per both sex categories were in the class R (15.4%). The carcass fatness assessment (*Table 2*) showed that the carcasses obtained from bulls were mostly average (10 or 76.9% in class 3) and low fat (3 or 23.1% in class 2) whereas the carcasses of heifers were characterized by clearly higher fat deposition (7 or 53.8% in class 3 and 6 or 46.2% in class 4). This findings, although limited due to small number of graded animals, were in accordance to fatness class distribution pattern for bulls and heifers reported by *Žgur* and *Drobnič* (1998) and *Florek* and *Litwinczuk* (2002).

Figure 1

Carcass cuts and corresponding beef retail categories



The carcass weight did not follow consistently the conformation grades (*Table 1*). The highest mean value of HCW was recorded in the class U for the bulls (296.5 kg) while in the same conformation class the mean HCW of heifers was the lowest (252 kg). As regarding to the carcass fatness (*Table 2*), the higher fatness class was followed by higher carcass weight for both, bulls and heifers. Dressing percentage was similar for each sex and with an increase in the muscle and fat deposition the carcass dressing percentage increased. The highest mean DP values were for bulls carcasses classified as class E (57.79%) and 3 (57.28%) while that of heifers were in the class E (57.71%) and 4 (58.03%).

In general, the amount of covering and internal carcass fat were visibly higher for heifers than for bulls and this difference was clearly showed through a percentage of separated fat. The mean value of SFAT in the most frequent conformation class U (*Table 1*) was significantly higher (P<0.05) for heifers (5.73%) than for bulls (4.14%). Similar distinction in SFAT percentage between sexes was also found in the most representative fatness class 3 (*Table 2*; heifers - 5.45% and bulls - 4.41%). However, the difference was statistically non-significant. The SFAT percentage increased correctly with higher fatness class and better conformation grade, as conformation includes the visual assessment of the thickness of both muscle and fat depots in relation to the size of the skeleton (*Kallweit* and *Henning*, 1998). The results for carcass cooling loss were similar for bulls and heifers with decreasing tendency as carcass fatness increase.

As expected, the bulls exhibited longer carcasses and legs, as well as larger perimeters of leg than the heifers, in correspondence to their higher finishing and slaughter weight.

Table 1

Least square means and standard errors (LSM±SE) for carcass traits of young bulls and heifers as related to conformation class of EUROP system

Carcass traits	E U R O P conformation class					
	bulls			heifers		
	E (n=4)	U (n=7)	R (n=2)	E (n=4)	U (n=7)	R (n=2)
HCW (kg)	291.0±12.5^{ab}	295.1±9.5^{a}	276.5±17.7^{ab}	272.5±12.5^{ab}	252.0±9.5^{b}	269.5±17.7^{ab}
DP (%)	57.79±0.82	57.15±0.62	55.25±1.16	57.71±0.82	57.42±0.62	56.22±1.16
SFAT (%)	4.38±0.59^{ab}	4.14±0.44^{a}	2.54±0.83^{ac}	6.48±0.59^{b}	5.73±0.44^{bc}	6.31±0.83^{bc}
CCW (kg)	275.5±10.9^{ab}	279.1±8.2^{a}	260.5±15.4^{ab}	252.8±10.9^{ab}	234.3±8.2^{b}	249.5±15.4^{ab}
CCL(%)	0.89±0.22	1.32±0.17	1.79±0.45	0.77±0.22	1.28±0.17	1.18±0.32
CL (cm)	130.9±1.9	135.0±1.4	132.5±2.6	129.6±1.9	128.8±1.4	133.3±2.6
LL (cm)	40.1±0.9	41.4±0.7	40.0±1.3	39.4±0.9	39.9±0.7	40.0±1.3
PL (cm)	120.8±2.2^{ab}	121.2±1.7^{a}	115.5±3.1^{ab}	114.5±2.2^{ab}	112.9±1.7^{b}	114.3±3.1^{ab}
MC (%)	43.55±0.56	43.21±0.37	42.92±0.65	44.29±0.48	43.71±0.37	43.63±0.65
Tissues:						
M (%)	69.38±0.83^{a}	70.97±0.63^{a}	70.75±1.18^{a}	65.43±0.84^{b}	68.04±0.63^{ab}	67.10±1.18^{ab}
B(%)	16.04±0.39	16.13±0.30	17.64±0.55	15.85±0.39	16.44±0.30	16.37±0.55
F(%)	8.07±0.91^{a}	7.06±0.68^{a}	6.44±1.28^{a}	12.25±0.91^{b}	10.03±0.68^{ab}	9.19±1.28^{ab}
T(%)	6.41±0.55	5.85±0.42	5.14±0.78	6.61±0.55	5.50±0.42	7.34±0.78
Meat Cat.:						
0 (%)	1.95±0.08	2.07±0.06	1.96±0.12	1.95±0.08	2.13±0.06	1.90±0.12
I (%)	30.51±0.43	30.36±0.32	29.74±0.61	30.60±0.43	30.62±0.33	30.01±0.61
II (%)	23.25±0.30^{ab}	23.68±0.22^{a}	23.66±0.42^{ab}	23.17±0.30^{ab}	22.65±0.22^{b}	23.17±0.42^{ab}
III (%)	44.29±0.44	43.89±0.33	44.64±0.62	44.28±0.44	44.60±0.33	44.92±0.62

^{a,b,c} Means with different letter within rows differ significantly at P<0.05.
HCW–hot carcass weight, DP–dressing percentage, SFAT–separated fat, CCW–cold carcass weight, CCL–carcass cooling loss, MC–Milanese cut, CL–carcass length, LL–leg length, PL–perimeter of leg, M–muscle, B–bone, F–fat, T–tendon, Meat Cat.–meat category.

The most prominent differences (P<0.05) were found for PL measurements within the conformation class U (*Table 1*) and fatness class 3 (*Table 2*), which were 121.2 and 120.3 cm in bulls and 112.9 and 111.4 cm in heifers, respectively. The better conformation and lower fatness class indicated higher share of ¨Milan cut¨ in both sex categories. The mean MC % in the bull carcasses (*Table 1*) ranged from 42.21 in class R to 43.55 in class E, while in the heifers carcasses percentage of MC were somewhat higher with the range from 43.63% (class R) to 44.29% (class E). As related to fatness classification (*Table 2*), the results for share of MC in the carcasses were: 42.98% in class 3 and 43.47% in class 2 for bulls and 43.67% in class 4 and 44.08% in the class 3.

The carcass tissue composition determined by dissection of the right half of the carcass showed, in general, the higher share of muscle and lower share of fat in the bulls than in the heifers carcasses. The mean values of muscle content (M %) for conformation classes E, U and R (*Table 1*) for bulls were 69.38, 70.97 and 70.75%, respectively. Whereas the corresponding values of M % for heifers were 65.43, 68.04 and 67.10%, respectively. The differences between sexes, however, were not statistically significant, except for the heifers in the class E.

Table 2

Least square means and standard errors (LSM±SE) for carcass traits of young bulls and heifers as related to fatness class of EUROP system

Carcass traits	E U R O P fatness class					
	bulls			heifers		
	2 (n=3)	3 (n=10)	4 (n=0)	2 (n=0)	3 (n=7)	4 (n=6)
HCW (kg)	272.7±12.2^{ab}	296.5±6.7^{a}	-	-	246.6±8.0^{b}	277.8±8.6^{ab}
DP (%)	56.31±0.94	57.28±0.52	-	-	56.72±0.62	58.03±0.67
SFAT (%)	2.49±0.55^{a}	4.41±0.30^{b}	-	-	5.45±0.36^{b}	6.75±0.39^{c}
CCW (kg)	259.0±10.9^{ab}	280.0±6.0^{a}	-	-	230.3±7.1^{b}	256.3±7.7^{ab}
CCL(%)	1.50±0.35	1.16±0.16	-	-	1.18±0.19	1.02±0.20
CL (cm)	136.7±2.2	132.4±1.2	-	-	129.8±1.4	129.7±1.5
LL (cm)	41.5±1.0	40.6±0.6	-	-	39.4±0.7	40.3±0.7
PL (cm)	120.0±2.4^{a}	120.3±1.3^{a}	-	-	111.4±1.6^{b}	116.2±1.7^{ab}
MC (%)	43.47±0.57	42.98±0.34	-	-	44.08±0.41	43.67±0.40
Tissues:						
M (%)	71.46±1.01^{a}	70.15±0.55^{ab}	-	-	67.85±0.66^{bc}	66.21±0.71^{c}
B(%)	16.24±0.45	16.36±0.25	-	-	16.73±0.30	15.68±0.32
F(%)	6.17±0.93^{a}	7.61±0.51^{ab}	-	-	9.28±0.61^{b}	12.10±65^{c}
T(%)	6.13±0.71	5.85±0.39	-	-	6.14±0.46	6.11±0.50
Meat Cat.:						
0 (%)	2.01±0.09^{ab}	2.02±0.05^{ab}	-	-	2.17±0.06^{a}	1.90±0.06^{b}
I (%)	30.65±0.48	30.21±0.26	-	-	30.73±0.31	30.28±0.34
II (%)	23.20±0.34^{ab}	23.65±0.19^{a}	-	-	22.76±0.2^{b}	23.04±0.24^{ab}
III (%)	44.14±0.50	44.13±0.27	-	-	44.34±0.33	44.79±0.35

^{a,b,c} Means with different letter within rows differ significantly at P<0.05.

HCW–hot carcass weight, DP–dressing percentage, SFAT–separated fat, CCW–cold carcass weight, CCL–carcass cooling loss, MC–Milanese cut, CL–carcass length, LL–leg length, PL–perimeter of leg, M–muscle, B–bone, F–fat, T–tendon, Meat Cat.–meat category.

Unexpectedly, the lowest share of muscle for both, bulls and heifers, was determined in carcasses classified as class E (the highest conformation score). As regards fatness class (*Table 2*), the M % varied more consistently and with the decreasing fatness grade the share of muscle in the carcass clearly increased. The mean M % of bulls in the fatness class 2 (71.46) were significantly higher (P<0.05) than in the class 3 (67.85) and class 4 (66.21) for heifers, whereas mean M % of bulls in class 3 (70.15) was significantly higher than M % in the class 4 for heifers. The opposite trend was apparent for carcass fat content which increased with better conformation grade. The mean F % for conformation classes E, U and R (*Table 1*) for bulls were 8.07, 7.06 and 6.44%, whereas the corresponding values of M % for heifers were 12.25, 10.03 and 9.19%, respectively. F % was not significantly different between sex groups, except for the heifers in class E. Expectedly, the F % increased with higher fatness grade (*Table 2*). The mean F % of heifers in the fatness class 4 (12.10%) were significantly higher (P<0.05) than in the class 3 (9.28%) for the same gender group and class 3 (7.61%) and class 2 (6.17%) for bulls group. Whereas the mean F % of heifers in class 3 (9.28) was significantly higher (P<0.05) than F % in the class 2 for bulls. Regarding bone (B %) and tendon (T %) percentage, no pronounced differences were observed between sexes within EUROP classification grades (*Table 1* and *2*). The mean values of the share of the most valuable beef cut: tender loin or beefsteak (0-category) and leg (I-category) were not significantly different between sexes within the conformation classes (Table 1). As related to fatness classes (*Table 2*), the significant difference between class 3 and class 4 was observed in the per cent of 0-category beef for heifers (2.17% and 1.90%, respectively). The lowered share of most valuable cuts in the heifers carcasses classified as fatness class 4 class was also reported by *Wajda* and *Daszkiewicz* (2002). The mean share of II-category beef in the carcasses classified as U was significantly higher (P<0.05) for bulls (23.68%) than for heifers (22.65%). Significant difference in per cent of II-category meat was also determined between bulls and heifers carcasses classified as fatness class 3 (23.65% and 22.76%, respectively). The results for mean share of III-category beef were similar for both, bulls and heifers carcasses.

CONCLUSIONS

The EUROP classification of ¨baby beef˙ carcasses showed a favorable conformation of both, young Simmental bulls and heifers with about one third carcasses graded as highest E class. The heifers fatness classification, however, was less favorable and almost half of heifers carcasses were classified as high fat and thus less valuable. The heifers over fatness was confirmed by high amount of trimmed fat and higher share of fat tissue and lower share of muscle than bulls after carcass dissection. The lowest proportion of muscle in the carcasses classified as most valuable E class imply a need for a additional improvement of assess practice as assessment is performed visually and the accuracy of classification largely depend on classificator experience. The differences between bulls and heifers in dressing percentage, carcass cooling loss, shares of Milanese cut, bone and tendon tissue as well as shares of cuts of different beef retail categories in the carcass were relatively small. This findings, however, need to be confirmed in the further investigations with larger number of ¨baby beef˙ cattle.

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Examination of suckling frequency in beef cattle populations

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ABSTRACT

This paper was carried out to study the connection between the mother cows (sucklers) and their calves during the lactation period, by examining suckling as a form of ethological behaviour. The daily suckling frequency (NSM) observed in two beef populations, we constructed suckling curves depends from some measured variables. The dam's genotype, the age of cows, the sex of calves, and some environmental agent (e.g. temperature) were tested as factors to influence the suckling frequency of calves. Regressions were done between the NSM and the weaning weight of calves.
(Keywords: suckling frequency, beef cows and calves, Hereford, Red Angus)

INTRODUCTION

It is reasonable to assume that the knowledge of suckling behaviour contributes to optimal management of beef cattle. However there is little information about suckling behaviour of beef cattle breeds and about those effects which are relation to the suckling frequency. The differences in suckling behaviour seem to be produced by a complex combination of genetic and environmental factors, which result in a particular behavioural relationship within mother and offspring pairs. The aim of this study was to describe the suckling behaviour - investigated two livestock.

Review of relevant literature

In the beef cattle production there is a big importance to calf-rearing ability, this property affects the benefit of these branches. The index of this ability, is the weaning weight corrected to 205 days, and its heritability is moderate (h^2=0,30–0,35). The genetic influence dissolved two parts: the additive genetic effect, and the maternal genetic effect. The second one means the influence of mother's genotype to the maternal traits, which affects the vitality and growth of calves (e.g.: easy calving; milking ability) (*Cameron*, 1997). Consequently, the environmental factors comprising a total of 60–70% from the phenotypic variance. The environmental effects were separated also two parts. Maternal effect origins from environment, and other environmental effects. It is well known that we called the maternal effect origins from environment, which inherit from year to year, and from calving to calving by beef cows (*Lengyel*, 2005). Some traits to be ranked here by authors, as the nursing of newborn calves, the possibility of suckling to own calf, protection of calf, and the part of cows' milking ability influenced by environment. According to *Kovács* (2005) those complex forms of behaviour, which are in relation to the suckling comprising 2–7% from the phenotypic variance of weaning weight.

According to the references the grazing period depends on the yield of the grass. If the pasture stay in a good state the cows grazing in 4–5 period per a day. The late summer and autumn the number of period decrease but the grazing time should be longer inside a period. The grazing activity is strong in the early morning and during the evening hours. This grazing habit is very important in respect of calves, because they have only a chance to suckle their mother during the break of grazing (*Márton*, 2003).

Other authors published, that the suckling frequencies of calves rise after driving, during the midday rest and evening hours (*Czakó*, 1978).

Usually, the newborn calves suckle 5–7 times per day (*Hafez* and *Lineweaver*, 1968). The number of suckling decreased in relation with the age, but beef calves suckle more because the milkmass of cows decreased during lactation (*Hafez*, 1975). According to *Houpt* and *Wolski* (1982) the beef calves suckling 3–5 occassions per day till weaning. The most frequent suckling period is at dawn, but following periods separated between 9.00–11.00; 15.00–18.00 and 22.30–01.00 (*Walker*, 1962). Others described that the number of suckling fall to daylight a large extent (*Shake* and *Riggs*, 1969). *Enyedi* and *Szuromi* (1991) separated 5 suckling periods during day-time and one more typically period during night from each other in summer. The suckling frequencies were the most proportion at midnight and after 5.00 a.m. in the morning, when more of the 50 percent of calves suckle.

Vandenheede et al. (2001) examined the relationship between the calf and cow after the Caesarean section and they established that the maternal behaviour was influenced by parity of cows. The first calvers seem to be poor as nursing, compared the oldest ones. In the first experiment we can found that the first calvers suckling more, but allosuckling also were observed in that group. Alien calves should be suck to first calvers, that these cows not sure the best for nursing. In addition to *Stookey* (1997) whether the cows allow all of the calves to suckle them, only the oldest calves would survive the lactation period.

According to *Lidfors* and *Jensen* (2003) calf-cow pairs spent more time together if the calf was female and if the weaning weight was smaller.

José et al. (2006) three behavioural traits were considered: number of suckling (NSM), duration of each suckling (DSM) and total suckling duration (TSD). Allosuckling was not observed. The calves suckled at any time during the daylight, and the overall means were NSM=2.57±0.05 meals/12 h; DSM=9.25±0.11 min., and TSD=23.76±0.47 min/12 h. There was an effect of dam's breed on NSM and DSM. The age of calf had significant effect on all traits. Males averaged higher NSM and TSD (2.60±0.03 and 25.05±1.37 min/12 h) respectively.

MATERIALS AND METHODS

Our investigations were done in different parts of the country, at Balatonfenyves (Hubertus Ltd.) and at Mezőfalva (Agricultural Cooperative) in the years 2002–2003 and 2005.

In Balatonfenyves result of the substitution crossing different genotype (Hereford; Hereford x Red Angus F1 and R1) were observed. The experimental groups represents pure blood Hereford cows (n=12; 11) crossing Hereford x Red Angus F1 cows (n=12; 11) and crossing Hereford x Red Angus R1 cows (n=10; 10) with their calves, in 2002 and 2003, respectively. The ethological investigation were done both year, 5–5 occasions (experimental day) in August.

In Mezőfalva the researching population contains three different aged dam's group, but genotype of these cows were similar to each other (Hereford x H. Simental F_1). First calvers were taken to the first group, second calvers were put in to the second group, and the oldest cows were taken to the third group (n=10; 10; 10), respectively. The researching period contains 10 experimental days in two terms (July and August).

On the experimental days the 24 hours long observation were applied (00.00–24.00.) During this time we observed the realization of suckling according to in every hours.

The influence of the two main factors (genotype and age) was tested by a nonparametric (Chi-square) test in every interval. Further genetic and environmental effects to the suckling frequency were examined by One Way Analysis of Variance. To clear up the relationship between measured valuables were used the linear regression. The statistical analyse were done by the software of *Microsoft Excel*, and *SPSS* 11.5 under the *Windows*.

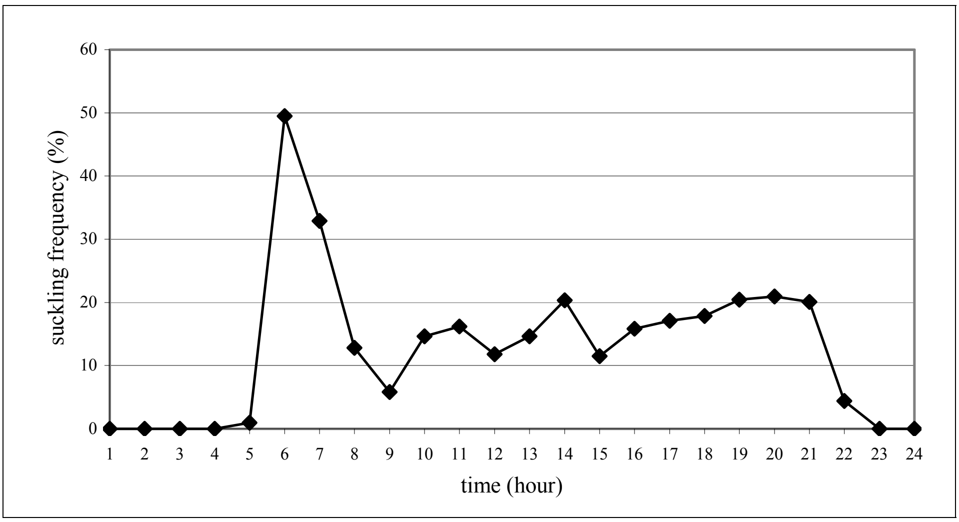
The meteorological data were collected (temperature; air pressure and rainfall) from the Countryside Meteorological Service of Siófok.

RESULTS AND DISCUSSIONS

In the first figure can be shown the suckling frequency in Balatonfenyves during a day without treatment. The average of number of suckling (NSM) was 3.06/day.

Figure 1

Change of suckling frequency during a day (n=330/hour)
– Balatonfenyves –

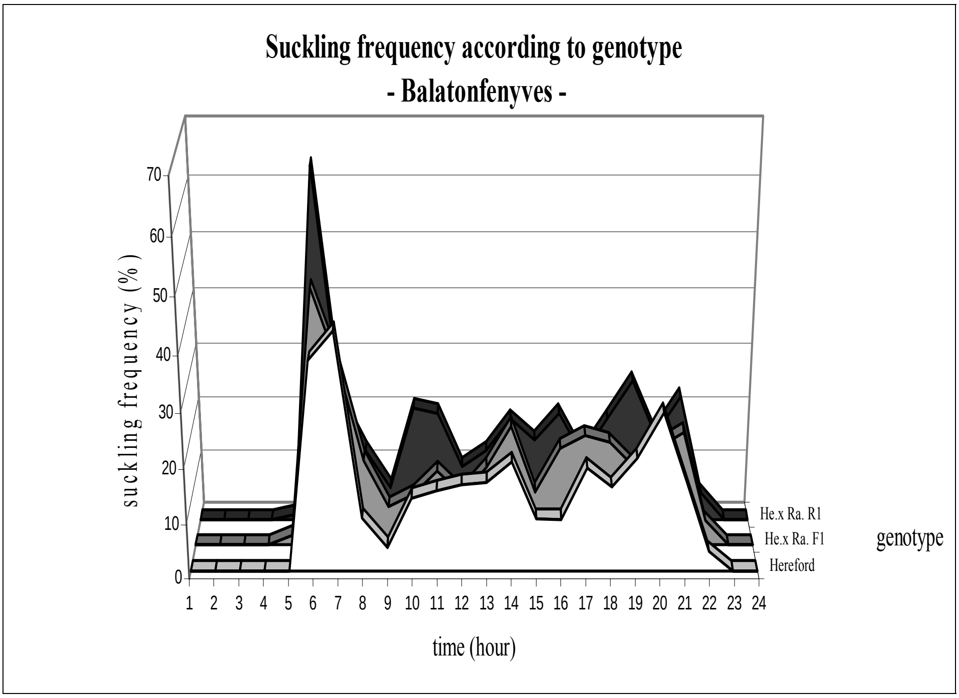


The suckling frequency rose after the animals woke up (5.00), and their (daily) peak reached between 5.30–6.30 a.m. After 8.00 a.m. the number of suckling decreased strongly. About at 9.00 a.m. the calves and the cows grazed, uniformly. We registered another two peaks duration of 10.00–11.00 a.m., and 13.00–14.30 p.m.. Afternoon, between 14.30–15.30 we observed a second grazing period with the respect of cows. After the nadir (around 15.00 p.m.) the suckling frequency increased slowly from 15.30 till at late evening hours (21.00). The ratio of suckling was about 20% during this duration. The suckling and the grazing were done simultaneously. We agree with *Walker* (1962), who said that the most frequent suckling period is at dawn, and *Czakó* (1978), who described that the suckling frequency rise after driving, during the midday rest and evening hours. At night no suckling were detected - correspondingly to *Shake* and *Riggs* (1969).

Investigated the suckling frequency regard to genotype, we got a significant difference (P<0,01). The Hereford x Red Angus R_1 cows suckle their calves much more (NSM=3.24/day), than the F_1 genotype or pure Hereford (NSM=2.98/day). The shape of suckling curve showed four peaks (R_1), opposite the other two genotypes (three peaks) (*Figure 2*).

Figure 2

Suckling frequency according to genotype
- Balatonfenyves -



José et al. (2006) also published that dam's breed was an effect on NSM. Because the R_1 genotype were first calvers simultaneously, the two effects (genotype and age) to separate from each other, was difficulty. Thus we decided, that the effect of dam's age should be examine independent from genotype. This purpose was appointed in the second research (Mezőfalva). *Table 1.* shown that there was not significant difference between the different age of dam.

Table 1

Influence of dam's age to the daily number of suckling (NSM)

age of dam	n	$\bar{x} \pm s$	source	sum of squares	df	mean square	F
1st calvers	100	3.72±0.73	age	0.26	2	0.13	0.238
2nd calvers	100	3.74±0.70	error	161.99	297	0.545	P
3rd calvers ≤	100	3.79±0.78	total	162.25	299		0.788

Respecting the number of suckling we got similar results with *Houpt* és *Wolski* (1982) who described that the NSM was 3–5/day. We examined is there any relationship between the sex of calves and the suckling frequency. Our results were shown in the *Table 2*.

Table 2

Influence of the sex of calves to the daily number of suckling (NSM)

place	source	sum of squares	df	mean square	F	P
Balaton-fenyves	sex of calves	0.0097	1	0.0097	0.084	0.773
	error	7.428	64	0.116		
	total	7.438	65			
Mezőfalva	sex of calves	3.333	1	3.333	0.935	0.342
	error	99.867	28	3.567		
	total	103.200	29			

There wasn't got significant difference between the variables independent from the experimental place. According to *José et al*. (2006) described that bull calves averaged higher NSM. On the other hand, *Lidfors* and *Jensen* (2003) calf-cow pairs spent more time together if the calf was female and if the weaning weight was smaller.

Result from the above mentioned fact, we examined the connection between the suckling number of calves and their weaning weight. The results of linear regressions were shown on the *Table 3*.

Table 3

Relationship between the weaning weight and the number of suckling (NSM)

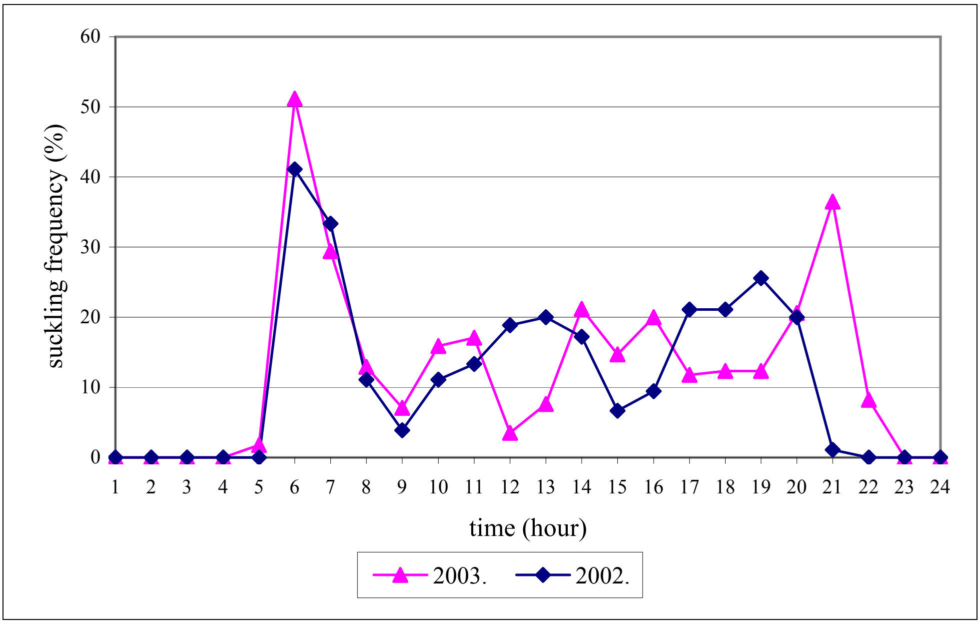
place	source	sum of squares	df	mean square	F	P
Balaton-fenyves	regression	0.315	1	0.315	2.816	0.098
	error	7.053	63	0.112	R=0,207	
	total	7.369	64		R^2=0,043	
Mezőfalva	regression	1.104	1	1.104	0.303	0.587
	error	102.096	28	3.646	R=0,103	
	total	103.200	29		R^2=0.011	

We got a slack and an obscure relationship with the two measured variables independent from the experimental place.

Relationship between the meteorological data (mainly the temperature) and the suckling frequency also were investigated. We got a significant difference (P<0.01) between the years (2002 and 2003) in Balatonfenyves (*Figure 3*) and the months (July and August) in Mezőfalva (*Figure 4*).

Figure 3

Change of the suckling frequency according to years
- Balatonfenyves -

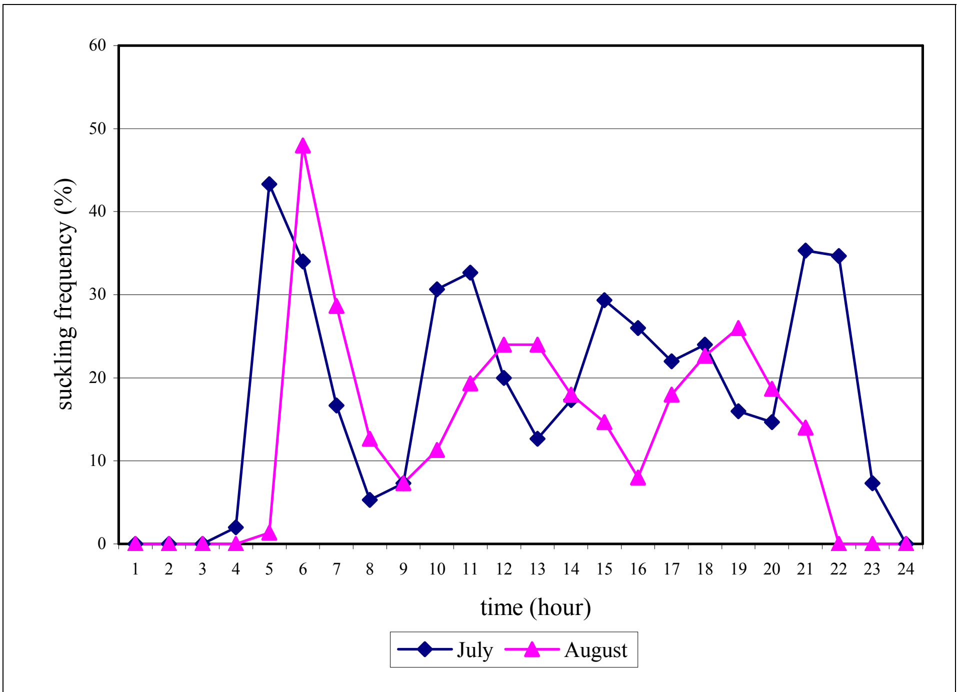


In duration of experiment the averaged daily temperature was 20.91 ºC in 2002., and 25.17 ºC in 2003. The high temperature divided two parts at the second suckling period about noon, and the number of suckling also increased (NSM were 2002=2.91; 2003=3.22).

Difference between the experimental days was analyzed only relation with year (Balatonfenyves) or month (Mezőfalva) in both cases (interaction). But while in Balatonfenyves the temperature stayed in background of difference, till in Mezőfalva may be the age of calves was responsible to the significant difference (NSM were July=4.33; August=3.16). The tendency of NSM relation to the months (Mezőfalva) similar with *Hafez* (1975), who published, that the number of suckling decreased in the respect with age. According to *José et al.* (2006) the all measured traits (e.g. NSM) were affected by the age of calf. There was not any connection between the change of the daily temperatures and the suckling frequency. That means when we progress to smaller interval (year/month→day→hour) decrease and at last leave off the direct influence of temperature to the suckling frequency. Strongly probable, that the temperature sensation stay at the background of this fact. Besides of actual (air) temperature the intensity of sunshine also plays role in hot sensation. It is not coincidence, that the resting of animals were observed in the early afternoon, but the peak of daily temperature was at 18.00 p.m. (2003=30.5 ºC).

Figure 4

Change of suckling frequency according to months
- Mezőfalva -



CONCLUSIONS

The daily suckling frequency observed in two beef populations. Number of suckling (NSM) increased after the animals woke up, and their (daily) peak reached in the morning hours. Smaller peaks were registered about at noon and during the evening hours. At night no suckling were detected. In Balatonfenyves genotype of the dam significantly affected ($P<0.01$) the suckling frequency. Because the small interaction between the dam's genotype and their age, we examined the influence of cows' age, in a separate trial (Mezőfalva). According to our results the age of the dam did not affect the suckling frequency significantly ($P>0.05$). NSM was not affected by sex of calves and there was low correlation between the NSM and the weaning weight of calves. Besides, difference between the experimental days was analyzed only relation with year (Balatonfenyves) or month (Mezőfalva) in both cases ($P<0.01$) (interaction).

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SECTION 3

HORSE, SHEEP AND GOAT BREEDING

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Genetic parameters of racing performance on Thoroughbred horses in Hungary

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ABSTRACT

The study aimed the estimation of variance components of racing ability traits in Thoroughbred horses. Collected data consisted of 1486 2–3–4-years-old general handicap weights (GHCP), 30 807 runs, 20 040 placings at finish, 16 605 earnings (log) won by 1 890 horses running in 3 316 races over the period of 1996–2004. Age of horses ranged from 2 to 12 years, and the distances varied from 1 000 to 3 200 m. Variance components were estimated by the residual maximal likelihood (REML) method. Statistical analysis accounted for fixed effects of year, age, race, sex, weight carried, trainer, jockey, ground and distance, and for the random effects of jockey, permanent environment, and additive genetic effects. Pedigrees were at least six generations deep. When trainer or rider effect was excluded from the model, heritability coefficients for earnings were 0.109 and 0.108 (repeatability 0.224 and 0.227), heritability for placings at finish were 0.059 and 0.059 (repeatability 0.142 and 0.14 respectively). Estimated heritabilities on the hendicap weights were high, 0.562 for the three-years-old, 0.661 in the and 0.663 for the four-year-old horses respectively.
(Keywords: thoroughbred, animal model, flat races, genetic parameters)

INTRODUCTION

Estimation of genetic parameters for handicap weight in the Hungarian Thoroughbred population has been reported by *Hecker* (1975) and *Bodó* (1976). These estimates were based on the paternal half sib or offspring-dam regression. During the last three decades no genetic parameters were estimated in this Thoroughbred population. The aim of this study was to provide genetic parameters and breeding value predictions on all Thoroughbred horses that participate at the Hungarian racetracks in flat races. This paper is the first of the series planned to be published and provides a detailed description of the data and of estimation of genetic parameters on racing performances measured by earnings and ranks.

Racing ability is the main selection criterion in Thoroughbreds. Breeding value estimation for racehorses also based on the racing ability in several countries (*Arnason et al.*, 1994; *Tavernier*, 1990). In Hungary the selection decisions based on pedigrees and sire statistics rather than on breeding values. Genetic-parameter estimates of the horse applying REML have described by *Huizinga et al.* (1989). Unfortunately there is no genetic parameter estimation carried out by the REML method in Hungary so far. Racing performance has also never been measured by the earning and ranking criteria.

The selection of Thoroughbreds is based on their racing performance usually on flat races. Most of the authors measure performances by earnings and ranks (*Hintz*, 1980; *Langlois*, 1980; *Langlois et al.*, 1996; *Sobczynska* and *Lukaszewicz*, 2003; *Langlois* and *Blouin*, 2004). In most cases a mathematical transformation is needed (*Langlois*, 1975). Earning and mathematical transformations of earnings (log of earning per start, log of annual earnings) have been discussed in several studies (*Hintz*, 1980; *Langlois*, 1980*; Langlois* and *Blouin*, 2004). Ranks also can be a measurement of performance (*Langlois*, 1980; *Langlois et al.*, 1996). *Sobczynska* and *Lukaszewicz* (2003) used square root of the finishing position for Arab horses in Poland. Heritabilities were 0.18 (repeatability 0.34) for the square root of ranks in Poland.

The aim of the authors was to estimate genetic parameters of racing performance measured by handicap weights, earnings and ranks in the Hungarian Thoroughbred population.

MATERIALS AND METHODS

There are two types of horse racing in Hungray. These are Thoroughbred flat races (99%) and Thoroughbred jumping races (0.6% hurdle and 0.4 % steeplechase races). Only the first type was considered in this study. Data on placings at finish and money prizes from 1996–2004 comprised 30 807 runs for 1890 horses competing in 3316 races. 30 614 race results were used during the analysis and 193 of them were excluded because of a racing accident or disqualification. The general handicap weights of 1485 animals according to three different age groups of 2, 3 and 4 year olds were 705, 1018 and 543 respectively. Horses were progeny of 311 sires and 512 dams, ages ranged from 2 to 12 years, race distances were 900 to 3200 meters. The pedigree information covered at least 6 generations and the total number of animals in the pedigree was 14 257. *SAS 9.1* (2004) software was used for data preparation and analysis of variance. The variance components were estimated by the REML method using VCE 4.2.5 (*Groeneveld*, 1998) software. The statistical classification for earnings and rankings accounted for fixed effects of age, sex (geldings were classified together with horses), year, race, trainer, jockey (rider), weight carried, class of the race. Genetic parameter estimation was performed using five different model.

In the first model the effect of the jockey was considered as fixed, but in the second it was random. In the following models the effect of the trainer or jockey, or the class of the race were excluded.

The earnings were log transformed to achieve normal distribution. Horses without earnings (from the sixth place) get the half the earnings of the horse ranked one place higher. Transformation for ranks was necessary in order to use normalized measure of performance. We used the square root of the ranks. General Handicap weights were collected from the annuals for the examined years of 1998–2004. In these models the effect of the sex and racing year were considered.

RESULTS AND DISCUSSION

Table 1 gives the number of horses, the number of horses raced, sire numbers, race records per horse and horses per sire. The number of races was decreased due the problems around the Hungarian racing systems during the last few years. It is also caused lower number of horses in these years. In the contrast of this the number of sires is increasing because of the numerous yearlings and sire imports.

Table 1

Races run, horse numbers, sire numbers, horses raced per sire, race records per horse, and records per races in flat races from 1998 to 2004 in Hungary

Years	1998	1999	2000	2001	2002	2003	2004	2005
Races	443	399	360	324	316	305	316	
Horses	455	479	487	416	414	428	391	
Records/Horse	8.4	8.1	7.4	7.5	7.1	6.5	7.4	
Race records	3 825	3 876	3 605	3 102	2 944	2 779	2 902	30 807

In *Table 2* ratios of variance components relative to phenotypic variance are presented. The highest heritability (0.11) and repeatability (0.25) were obtained for log of earnings when trainer or jockey was excluded from the model. These results match closely the heritabilities published by *Chico* (1994), *Sobczynska* and *Lukaszevicz* (2004), *Svobodovaa* (2005), *Langlois* (1996). *Preisinger et al*. (1990) also reported that ignoring the trainer or the jockey effect in the model also caused increase heritability.

In the case of square-root of placings at finish lower genetic parameters were estimated as shown in *Table 3*. The results are lower than those Sobczynska and Lukaszevicz reported in Poland (2004), however our results on log of earnings are match to their finding. Ignoring of the trainer or the jockey effect caused increased heritabilities.

Table 2

Estimated genetic parameters (p.e.=permanent environment; h^2=heritability; r=repeatebility)

Traits	Log of earning			Rank		
	p.e.	h^2	r	p.e.	h^2	r
Jockey as fixed	0.177±0.016	0.093±0.017	0.270±0.033	0.089±0.013	0.050±0.014	0.139±0.027
Jockey as random	0.114±0.015	0.093±0.016	0.207±0.031	0.086±0.013	0.052±0.014	0.138±0.027
No trainer	0.115±0.017	0.109±0.018	0.224±0.035	0.083±0.013	0.059±0.013	0.142±0.026
No jockey	0.119±0.016	0.108±0.018	0.227±0.034	0.083±0.013	0.059±0.013	0.142±0.026

Estimated genetic parameters for handicap weights were high compared with other studies (*Schulze-Schleppinghoff et al*. 1985; *Dušek*, 1963, 1965; *More O'Ferral* and *Cunningham*, 1974; *Hecker*, 1975; *Bodó*, 1976; *Hintz*, 1980).

Table 3

Mean values, standard deviation and heritabilities of handicap weight in different age

Age	2	3	4
Effects in the model	- Year (F) - Age (F)	- Year (F) - Age (F)	- Year (F) - Age (F)
n	705	1018	543
mean	50.22	48.30	50.85
Standard deviation	9.55	13.48	13.70
Heritability	0.562±0.098	0.661±0.088	0.663±0.141

CONCLUSIONS

The estimated heritabilities of the two criteria (earnings and ranks) can be considered low. The log of earnings seems so to be the better measurement of racing performance. Estimations on general handicap weight for the two-, three- and four-year old horses were high. These results show that there has been a considerable overestimation of the additive genetic influence on race performance measured by general handicap weights.

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Behaviour of sheep in three different types of paddock in karst region of Slovenia

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ABSTRACT

Forty sheep and ten goats were reared on three different types of paddock (grass paddock, woody, and partly woody paddock). Behaviour of the ten marked sheep was observed during the time of day light (between 5 a.m. and 9 p.m.). Frequency of the following activities was monitored: grazing, drinking, salt consumption, aggressive behaviour, comfort behaviour and resting (in lying position, in standing position and resting in the flock). On average, the animals were grazing 10.5 hours per day. Circadian rhythm of grazing was different, depending on the type of paddock and on average daily temperature. Drinking frequency was very low. On average, each animal drank 0.99 times per day. Salt consumption, frequency of aggressive behaviour and comfort behaviour were most frequent in woody paddock. Two types of resting (in lying position and resting in the flock) had almost the same percent of the total resting time (46–47%), while the third type of resting (in standing position) had the lowest percent in all three types of paddock (about 7%).
(Keywords: small ruminants, sheep, animal behaviour, pasture)

INTRODUCTION

Due to the climate, geological and morphological conditions over 70% of agricultural land in Slovenia is classified as less favoured for agricultural production and karst occupies almost half of the territory (SURS, 2002). According to the *Cunder* (1998) between 120.000 and 150.000 ha of agriculture land was abandoned in last few years and they are already overgrown with shrub, trees and brushwood. The process is especially distinctive in the lower karst and high alpine region (*Cunder*, 1999), where use of mechanisation is difficult and a result is the land which is not adequately cultivated. Those areas are therefore appropriate for small ruminants, because maintains grasslands and prevents land from bush encroachment and fires.

For the better management of the sheep flocks and choice of the appropriate production methods conditions in the breeding environment has to be well-known. Even more important is to know the behaviour of animals in such conditions. In Slovenia it was tradition that a few goats in a flock of sheep were added. The aim of our study was therefore to observe the behaviour of sheep in a mixed flock with goats in three different paddocks in the karst region of Slovenia, to gain a firm knowledge on ethological traits of sheep.

MATERIALS AND METHODS

The experiment was undertaken during the August and September, on the hilly karst region (900–1000 m a.s.l.). Forty sheep and ten goats grazed together in three different types of paddock: grass paddock, woody, and partly woody paddock.

The total observation time was 12 days. Animals were observed for two days in each paddock with one rotation. Daily observation time was between 5 a.m. and 9 p.m. The animals were in the same paddock six days. During the experiment they were moved from one type of paddock to another due to lack of the feed. To assure that animals were adapted to new paddock, observation started third day after they were moved. For the purpose the ten sheep, approximately the same age, were marked. Every 5 min during 16-h observation time, grazing and resting (in lying position, in standing position, and resting in the flock) of the sheep was recorded. Drinking, salt consumption, aggressive behaviour, withdrawal and comfort behaviour were counted at appearance.

The average daily temperature of 12 recording days was 14.1 °C, with maximum air temperature 18.5 °C and minimum air temperature 9.2 °C. Observations were carried out directly from the observation point; a distance approximately 100–200 m away from the paddock using the binoculars. In woody and partly woody paddock the observer was inside the paddock. The animals were accustomed with the observer; therefore their attendance did not disturb them.

During observation data were written down and later on entered into the computer. They were analysed with the statistical package SAS/STAT. The general linear model (GLM) was used to determine the effects of normally distributed data. The daily values of data were tested for normality. Differences between the types of paddock were analysed using the estimate phrase of GLM procedure.

Activities like resting (in standing position and resting in the flock), salt consumption, drinking, aggression, withdrawal and comfort behaviour were not normally distributed. Those activities are presented and described briefly. Normally distributed data: grazing, resting (total) and resting in lying position were analysed with the GLM procedure, using a model which included the effects of paddock, day and animal. The average daily temperature was included as independent variable.

The following model was used to test various effects on grazing and resting:

$$y_{ijk} = \mu + C_i + D_j + Z_k + b_i \cdot (t_{ijkl} - \bar{t}) + e_{ijkl}$$

y_{ijk} : duration and frequency for i-paddock, j-day and k- average daily temperature,
μ : mean value,
C_i : effect of the type of paddock,
D_j : effect of the day,
Z_k : effect of the animal,
b : regression coefficient,
t_{ijkl} : average daily temperature mean value $\bar{t}$,
e_{ijkl} : difference.

RESULTS AND DISCUSSION

Grazing

Grazing is defined as the time spent each day in grazing activity, that is, the prehension and the mastification (*Woodward*, 1997). Animals were in the paddocks 24 hours per day. As shown in *Figure 1*, total grazing time between 5 a.m. and 9 p.m. was on the average 10.5 hours per day. *Lynch et al*. (1992) reported that the sheep grazed 8-9 hours per day and *Hecker* (1983) reported that the average grazing time per day was 9 hours with the maximum grazing time about 13 hours when feed supply was limited.

Usually grazing can occur at any time of the day or night but is most intensive in the morning and late afternoon until dusk.

As shown in *Figure 2*, we found differences in daily rhythm between different types of paddock. Furthermore, sheep grazed at the sunrise and just before the sunset. Similar findings were reported by *Lynch et al.* (1992) who explained that in continental areas grazing activity is concentrated to 4 hours after dawn and in the last 4 hours around sunset, but can easily start before dawn and extend long into the dark. The average daily temperature did not have any significant effect on grazing time. It only affected the circadian rhythm of grazing (*Figure 3*).

Sheep are typically classified as social animals. *Rook* and *Penning* (1991, cited by *Champion et al*., 1994) concluded that sheep tend to be synchronous in their start of the grazing bouts. The first day of our observation, grazing time was lower compared with grazing time in the second day of observation. *Hodgson* (1985, cited by *Woodward*, 1997) explained that the major factor affecting grazing time is the herbage availability. When the herbage availability is extremely low, it is common that grazing time increases.

Statistical analysis showed that the type of paddock significantly effected the duration of grazing (P<0.01). As shown in *Figure 1*, grazing was longer in grass paddock and shorter in woody and partly woody paddock. According to *Vidrih et al.* (1996) there are differences in herbage composition between types of paddock resulting in different duration of grazing time.

Figure 1

Grazing and resting time per animal per paddock

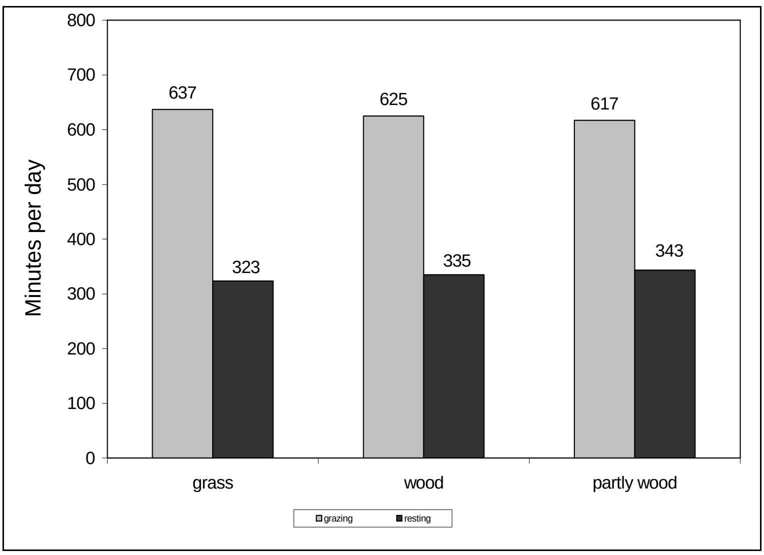


Figure 2

Daily grazing rhythm in grass, woody, and partly woody paddock

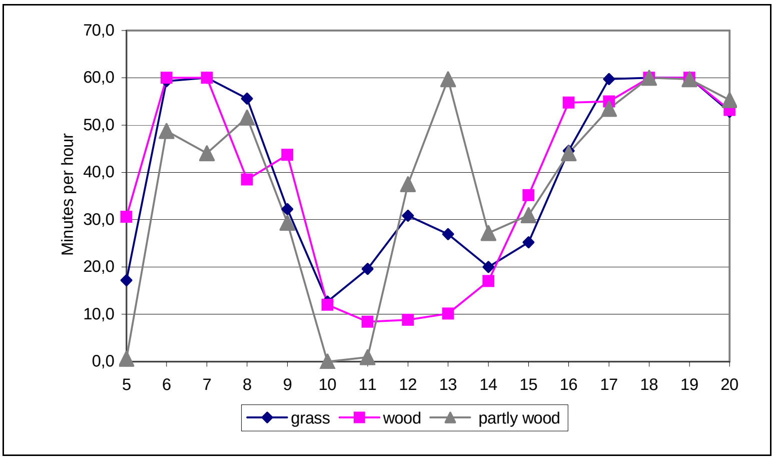
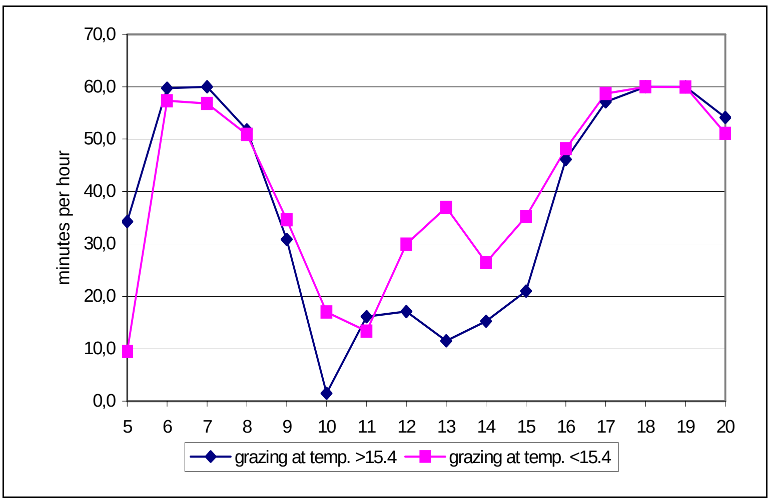


Figure 3

Grazing rhythm at temperatures above and below the average daily temperature



Resting

Resting in lying position and resting in the flock had almost the same percentage as total resting time (46–47%). As shown in *Figure 4*, the third type of resting in standing position had the lowest percent, only about 7% of the total resting time. Statistical analysis showed significant effect of the type of paddock on the resting time ($P<0.01$). The effect of the consecutive day after moving into another paddock on the resting time was also significant ($P<0.001$). During hot weather sheep were resting in the shade under the trees, except in grass paddock which was without a shade. Resting in the flock appeared to be most frequent. Sheep formed subgroups of different sizes.

In a sunny weather, sheep usually stretched their necks or even pushed their heads under the other animal's belly. *Lynch et al*. (1992) reported that during the summer period sheep preferred resting in the shade under the trees, and if no shade was available sheep spent many hours resting at the highest site of the paddock. As shown in *Figure 1*, total resting time was on average 6 hours per observation period of the day. *Lynch et al.* (1992) also reported that the total resting time was 10 hours per day. As shown in *Figure 5*, the daily rhythm of resting periods was converse to the daily rhythm of grazing (*Figure 2*).

Figure 4

The rate of different type of resting in total resting time per paddock

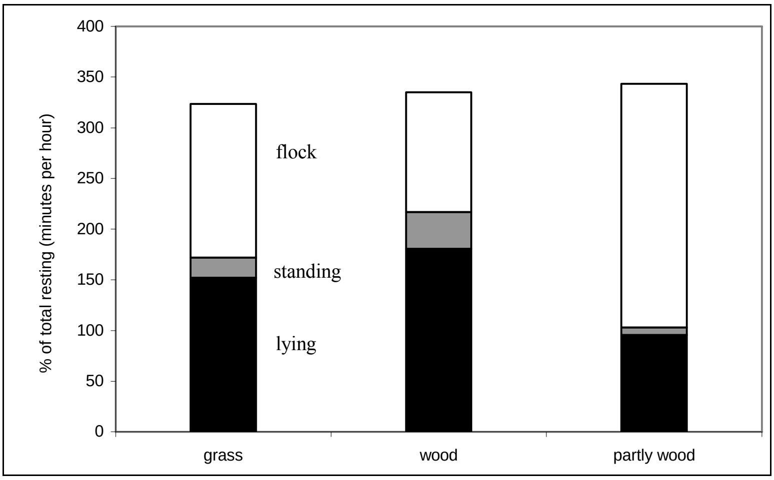
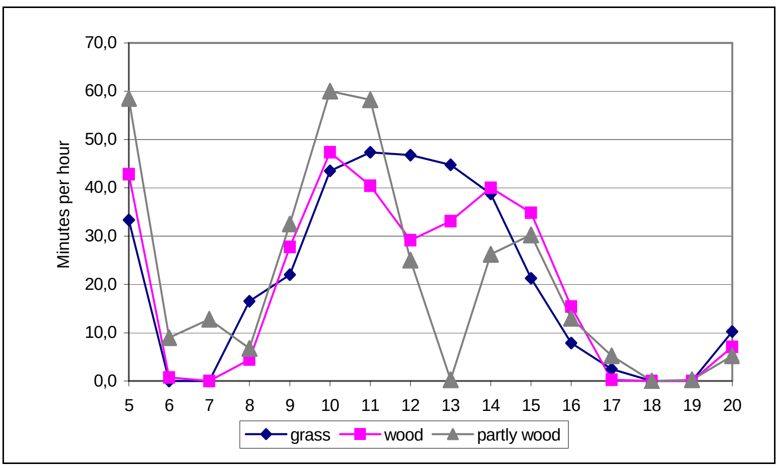


Figure 5

Daily resting rhythm in grass, woody, and partly woody paddock



Drinking

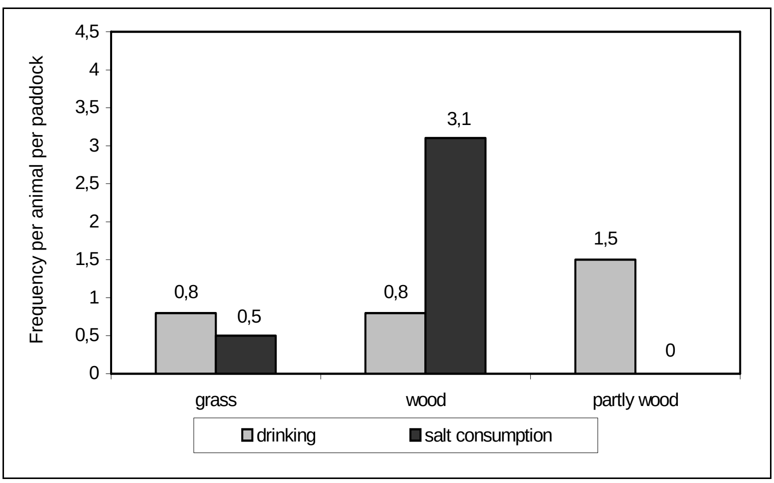
All animals had free access to water. We noticed that the animals tended to be synchronous in their going to the water supply. When one or two animals started to approach the water supply the other animals followed. Similar findings were reported for sheep by *Vidrih et al*. (1996) and explained that drinking is a group activity (alelomimetic behaviour). As shown in *Figure 6*, drinking frequency was low during the observation time. We noticed that during observation, between 5 a.m. and 9 p.m., some sheep did not drink water at all. *Hecker* (1983) and *Kermauner* (1996) reported that sheep may graze without having the access to water for a long period. *Lynch et al.* (1992) reported that during summer sheep should drink at least once a day. Otherwise, they tend to reduce the grazing time in the heat and increase grazing at night and early in the morning, when dew is on the grass. Due to our calculation the average drinking frequency was 0.99 per animal during our observation time. Drinking frequency was higher in the morning between 8 a.m. and 9 a.m., and in the afternoon between 3 p.m. and 7 p.m. Drinking frequency was also higher in partly woody paddock and lower in grass and in woody paddock.

Salt consumption

Animals had free access to the salt-lick. Frequency of the salt intake was higher in woody and lower in grass paddock. As shown in *Figure 6*, animals did not consume salt in partly woody paddock during our observation time. The salt intake frequency was higher in the morning between 6 a.m. and 8 a.m. and in the afternoon between 5 p.m. and 9 p.m. *Vidrih* (1996) analysed the leaves of the hazel and beech tree and found out that leaves contained 0.15–1.17 g sodium/kg of dry matter. Sheep under 50 kg of body weight need 1.5 g of sodium in dry matter for normal life. In woody paddock animals were chewing the bark of a tree or wood itself. *Kermauner* (1996) reported that chewing was a consequence of the lack of sodium.

Figure 6

Frequency of drinking and salt consumption per animal per paddock

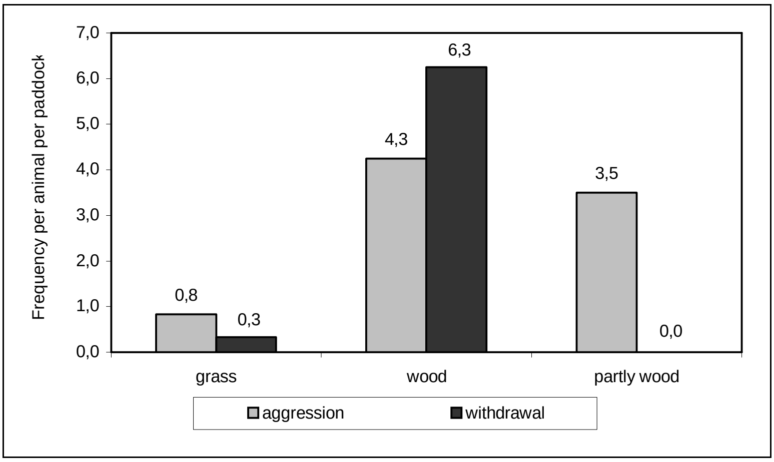


Aggressive behaviour and withdrawal

The frequencies of aggressive behaviour and withdrawal were very low. More frequent was the aggressive behaviour around salt and drinking trough. In woody paddock aggressive behaviour was higher probably because of the bushes (*Figure 7*). When animals (mostly goats) reached branches and pulled them down, all animals wanted to eat. Such situations were in most cases the reason for aggressive behaviour. Animals butted with the head into the side or rump of the other animal. In such situations the goats were more active, therefore increased withdrawals were noticed in sheep (*Figure 7*). Similar findings were reported by *Lynch et al*. (1992).

Figure 7

Frequency of aggressive behaviour and withdrawal per animal per paddock

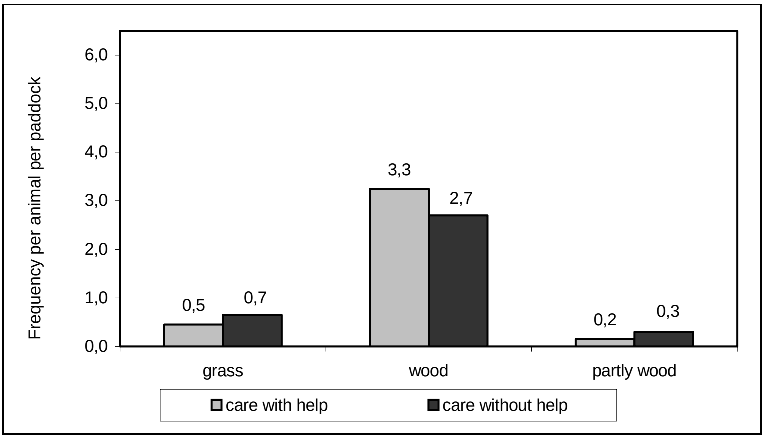


Comfort behaviour

Fraser and *Broom* (1990) explained comfort behaviour as: licking of the body, biting, skin care, rubbing of the horns, neck, muzzle, face and scratching with a leg. In partly woody and woody paddock they used trees and bushes as a help in comfort behaviour. For this purpose we placed a woody timber into grass paddock. As shown in *Figure 8*, frequency of selfcare with or without help was higher in woody paddock than in partly woody and in grass paddock. Animals took care of the neck, lateral and back side of the body with the help of trees and bushes. Similar findings were reported by *Fraser* and *Broom* (1990) who maintained, that animals took care of their bodies with the help when they could not reach the definite part of the body. These parts are often head, neck and hips which they care for with the help of rubbing them at a pillar, a tree, a door or a fence. Biting definite part of the body or scratching with a leg is considered as a care without any outside help, similar as was explained by *Fraser* and *Broom* (1990). Frequency of the care with help was 1.33 per day, and frequency of the care without help was 1.27 times per day in our case.

Figure 8

Frequency of comfort behaviour per animal per paddock



CONCLUSIONS

During the observation time between 5 a.m. and 9 p.m., sheep were grazing on average 10.5 hours per day. The type of paddock influenced the duration of grazing time and daily rhythm of the sheep. During the afternoon heat animals moved to the shade, if available. Shade should be provided if not available in the paddock. Drinking frequency was very low, only 0.99 per animal per day. Salt consumption frequency was the highest in woody paddock, which can be explained by the lack of sodium in the leaves and branches that are also often eaten. The aggression frequency was very low. The highest number of aggression and withdrawal was during the hustling for branches in woody and partly woody paddock. In the other paddock the aggression and the hustling appeared only around the salt lick. The frequency of comfort behaviour was the highest in woody paddock, despite the fact that the animals had wooden timber for this purpose in grass paddock.

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Estimation of daily milk yield from alternative milk recording schemes in dairy sheep

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ABSTRACT

The objectives of this study were to develop and compare different models for estimation of daily milk yield from alternative milk recording scheme (single morning and evening milking records). In this study, 3.000 individual test-day milk yield records from Central data base of Agricultural institute of Slovenia were used. Data were collected from April 1999 to September 2002 on 565 sheep reared on 10 family farms in Slovenia. Daily milk yield as well as daily fat and protein content were estimated by several statistical models. Determination coefficients of models for estimation of daily milk yield as well as daily protein were slightly lower when estimation was based on evening milkings, while the determination coefficients of models were slightly higher when daily fat content was estimated from evening milking. With the complexity of the models the amount of explained variance increases and the bias between true and estimated daily yields decreases. Inclusion of other effects in models, beside single milking weights, like effects of breed, lactation stage and number of liveborns, did not significantly increase the amount of explained variance, so the differences between models used for estimation were minor and statistically insignificant, therefore we would recommend use of model A in practice. That model included only partial milk yield as linear regression so, because of its simplicity, the implementation in routine work is simple. Because in present research the information of the interval between successive milkings, which is the most important effect in estimation of daily yields, was not available there is a need for further investigation in which we would be able to take that effect into account.
(Keywords: alternative milk recording scheme, daily milk yield, estimation, sheep)

INTRODUCTION

Milk recording provides collection of milk yield data required for herd management as well as for genetic evaluation of dairy animals. In the last decades, numerous milk recording schemes have been developed in many countries (*McDaniel*, 1969; *Wiggans*, 1981; *Rosati et al*., 1998; *Sanna et al*., 1998; *Drobnic et al*., 2000) with purpose of supplementation of the standard four-weekly testing scheme (A4) which is considered as the most expensive one. The alternative milk recording (morning or evening) testing scheme was designed to gain lower cost and to retain reasonable accuracy in daily milk yields prediction. When alternative milk recording scheme is used estimation of daily yield is necessary. For estimation of daily milk yield from single milking weights various models have been developed. Depending on the model, different factors that influence milk production were taken into account, like breed, parity, lactation stage, and the interval between successive milkings (*Hargrove*, 1994; *Cassandro et al*., 1995;

Klopčič et al., 2001). The milking interval was shown as the most important factor when daily milk yield is estimated from morning or evening milkings. Most of the studies on milk recording schemes were conducted on dairy cattle, but the same principles can also be applied to sheep. Currently, the common practice in estimation of daily milk yield from alternative milk recording schemes is simply doubling of the morning or evening yield which frequently results in biased estimates of the daily milk yield (*Jovanovac et al*., 2005). The objectives of this study were to develop and compare different models for estimation of daily milk yield from alternative milk recording scheme (single morning and evening milking records).

MATERIALS AND METHODS

In this study, 3.000 individual test-day milk yield records from Central data base of Agricultural institute of Slovenia were used. Data were collected from April 1999 to September 2002. During research, measurements were conducted on 565 sheep reared in 10 family farms in Slovenia. From all sheep 50.47% belonged to the Bovška breed, 20.32% belonged to the Improved Bovška breed, while 23.21% of all sheep belonged to the Istrian sheep. At each recording, milk yield was measured in the evening and in the morning. Daily milk yield was computed as evening plus morning measured yield. Initial time of current milking and initial time of previous milking was not registered, so the interval between successive milkings could not be calculated. For analysis of milk composition three samples were taken from each animal: one sample at each milking (evening and morning) and one proportional milk sample. Additionally, a linear regression of daily to evening or morning records was fitted in order to detect outliers. Residuals over three standard deviations were taken as outliers and deleted from data set. Variability of daily, morning and evening milk yield, as well as fat and protein content is reported in *Table 1*.

Table 1

Descriptive statistics for milk traits

Trait	Milk yield, ml			Fat content, %			Protein content, %		
	n	Mean	SD	n	Mean	SD	n	Mean	SD
Daily	2950	1155.13	603.99	2850	6.19	1.15	2870	5.39	0.68
Morning	2950	583.02	322.95	2850	5.92	1.22	2870	5.40	0.72
Evening	2950	570.31	304.72	2850	6.46	1.35	2870	5.40	0.69

Correlations between daily, morning or evening milk yield as well as fat and protein content are presented in *Table 2*. Evening milkings have lower correlations with daily yields than morning milkings. Obtained correlations are in agreement with those that are published (*Lee* and *Wardrop*, 1984; *Cassandro et al*., 1995; *Trappmann et al*., 1998; *Liu et al*., 2000). The correlation between daily and morning protein content is higher than correlation among daily and evening contents, while the correlation between daily and morning fat content is lower than correlation among daily and evening contents. The similar results were reported by *Klopčič et al.* (2003). The lowest correlation with its daily measurements has fat content measured on single milkings, which means that the accuracy of daily yield estimation from single milking will be lowest if estimation of fat content is observed.

Table 2

Correlations between daily, morning or evening milk yield, fat and protein content

Trait	daily – morning		daily – evening		morning – evening	
	r	p	r	p	r	p
Milk yield, kg	0.969	<0.0001	0.966	<0.0001	0.873	<0.0001
Fat content, %	0.891	<0.0001	0.913	<0.0001	0.627	<0.0001
Protein content, %	0.974	<0.0001	0.972	<0.0001	0.894	<0.0001

For statistical analysis the SAS/STAT package was used (*SAS* Institute Inc., 2000). Daily milk yield as well as daily fat and protein content were estimated by several statistical models (*Table 3*). Models were compared on the basis of the determination coefficient (R^2), variability coefficient for standard error (CV_e**)** and root mean square error (σ_e**)**. Differences between statistical models were tested according to *Mead* (1970).

Table 3

Statistical models for estimation of daily milk yield, fat and protein content

Model	df	Factors included in model								
		B	N	m	sl	sl^2	sl^3	$sl_{Wilmink}$	$sl_{Guo\text{-}Swalve}$	$sl_{Ali\text{-}Schaeffer}$
A	2			+						
B	3			+	+					
C	4			+	+	+				
D	5			+	+	+	+			
E	4			+				+		
F	6			+					+	
G	6			+						+
I	4	+		+						
J	7	+	+	+						

df–degree of freedom, B–breed, N–number of liveborn; m–morning or evening milk yield, sl–lactation stage, Wilmink–lactation stage as Wilmik curve (*Wilmink*, 1987), Guo-Swalve–lactation stage as Guo-Swalve curve (*Guo* and *Swalve*, 1995), Ali-Schaeffer–lactation stage as Ali-Schaeffer curve (*Ali* and *Schaeffer*, 1987).

RESULTS AND DISCUSSION

Determination coefficient (R^2), variability coefficient for standard error (CV_e**)** and root mean square error (σ_e**)** for models used to estimate daily milk yield from single milking weights are shown in *Table 4*. The model with the highest determination coefficient and lowest root mean square error fits the best to the data set. The amount of explained variance enhances with the complexity of the models. Determination coefficient (R^2) values for models based on morning milk yield ranged from 0.9533 in model A, which included only partial milk yield as linear regression, to 0.9546 in model D, F and G that included, beside partial milk yield as linear regression, also effect of lactation stage as cubic curve, as lactation curve by Guo and Swalve and as lactation curve by Ali and Schaeffer. Determination coefficients (R^2) are slightly lower when estimation is based on evening milkings which differ from the results obtained by *Klopčič et al*. (2001). These results indicate that estimation of daily milk based on morning milking will be more reliable than those based on evening milking, which is in agreement with published results (*Cassandro et al*., 1995; *Lee et al*., 1984; *Liu et al*., 2000; *Jovanovac et al*., 2005).

Table 4

Determination coefficient (R^2), variability coefficient for standard error (CV_e) and root mean square error (σ_e) for models used to estimate daily milk yield from single milking weights

Model	df	Morning milking			Evening milking		
		R^2	CV_e	σ_e	R^2	CV_e	σ_e
A	2	0.9533	11.2658	128.6690	0.9470	12.0024	137.2427
B	3	0.9545	11.1159	126.9939	0.9486	11.8108	135.0906
C	4	0.9545	11.1117	126.9451	0.9489	11.7880	134.8297
D	5	0.9546	11.1113	126.9412	0.9489	11.7898	134.8505
E	4	0.9545	11.1169	127.0051	0.9487	11.8102	135.0837
F	6	0.9546	11.1059	126.8798	0.9490	11.7743	134.6727
G	6	0.9546	11.1129	126.9593	0.9491	11.7691	134.6134
I	4	0.9541	11.1759	127.6424	0.9473	11.9720	136.8949
J	7	0.9541	11.1788	127.6732	0.9473	11.9671	136.8295

The bias in model A for estimation of milk yield, both from morning or evening milking was low (*Figure 1* and *2*). Higher values were slightly underestimated while the lower values of daily milk yield were slightly overestimated when model A was used for estimation.

Figure 1

Bias in model A for estimation of daily milk yield from morning milkings

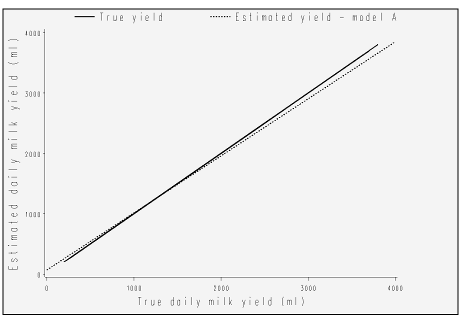
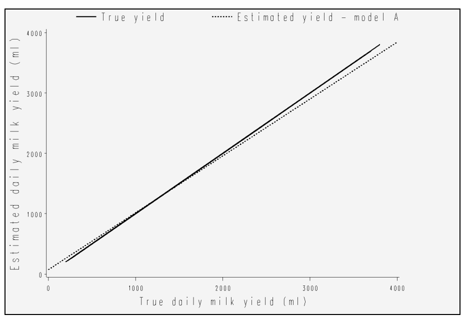


Figure 2

Bias in model A for estimation of daily milk yield from evening milkings



Determination coefficient (R^2) for models used to estimate daily fat content from morning milking is ranged from 0.8196 (model A) to 0.8369 (model G) (*Table 5*). Determination coefficients are slightly higher when prediction is based on evening milkings which was expectable because correlation between evening and daily fat content was higher than the correlation between morning and daily fat content. These results are in agreement with the those obtained by *Liu et al*. (2000).

Table 5

Determination coefficient (R^2), variability coefficient for standard error (CV_e) and root mean square error (σ_e) for models used to estimate daily fat content from single milking

Model	df	Morning milking			Evening milking		
		R^2	KV_e	σ_e	R^2	KV_e	σ_e
A	2	0.8196	7.8990	0.4881	0.8526	7.1100	0.4392
B	3	0.8360	7.5322	0.4655	0.8573	6.9980	0.4322
C	4	0.8362	7.5286	0.4652	0.8584	6.9730	0.4307
D	5	0.8367	7.5191	0.4646	0.8584	6.9728	0.4307
E	4	0.8364	7.5260	0.4651	0.8580	6.9830	0.4313
F	6	0.8367	7.5219	0.4648	0.8586	6.9694	0.4305
G	6	0.8369	7.5152	0.4644	0.8587	6.9660	0.4303
I	4	0.8281	7.7140	0.4767	0.8599	6.9352	0.4284
J	6	0.8284	7.7119	0.4766	0.8600	6.9371	0.4285

Figure 3

Bias in model A for estimation of daily fat content from morning milkings

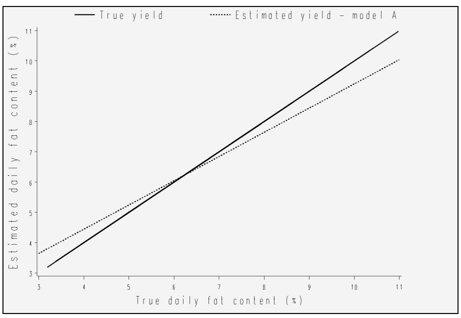


Figure 4

Bias in model A for estimation of daily fat content from evening milkings

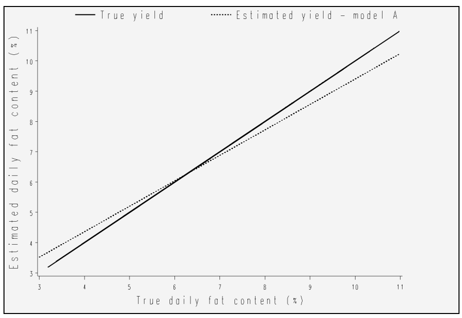


Figure 3 and *4* show bias in model A for estimation of daily fat content from morning or evening milkings, respectively. Lower values (<5%) of daily fat content were overestimated while the higher values (>8%) were underestimated when model A were used for estimation.

Table 6 shows determination coefficient (R^2), variability coefficient for standard error (CV_e) and root mean square error (σ_e) for models used to estimate daily protein content from morning or evening milking. Determination coefficient (R^2) for models used to estimate daily protein content from morning milking are ranged from 0.9643 in model A to 0.9646 in models F and G which included, beside partial milk yield as covariate, also effect of lactation stage as lactation curve by *Guo* and *Swalve* (1995) and as lactation curve by *Ali* and *Schaeffer* (1987). Determination coefficients are slightly higher when prediction is based on morning milkings which is in agreement with the results obtained by *Liu et al*. (2000) and which differ from results reported by *Klopčič et al*. (2003). The differences in accuracy between models were minor and statistically insignificant, both for estimation based on morning or evening milking.

Table 6

Determination coefficient (R^2), variability coefficient for standard error (CV_e) and root mean square error (σ_e) for models used to estimate daily protein content from single milking

Model	df	Morning milking			Evening milking		
		R^2	KV_e	σ_e	R^2	KV_e	σ_e
A	2	0.9643	2.3715	0.1276	0.9604	2.5107	0.1352
B	3	0.9644	2.3689	0.1275	0.9630	2.4279	0.1308
C	4	0.9645	2.3672	0.1274	0.9630	2.4281	0.1308
D	5	0.9645	2.3675	0.1274	0.9630	2.4275	0.1307
E	4	0.9645	2.3673	0.1274	0.9630	2.4283	0.1308
F	6	0.9646	2.3640	0.1272	0.9631	2.4266	0.1307
G	6	0.9646	2.3660	0.1273	0.9630	2.4279	0.1308
I	4	0.9645	2.3689	0.1275	0.9607	2.5031	0.1348
J	7	0.9645	2.3691	0.1275	0.9607	2.5029	0.1348

Figure 5

Bias in model A for estimation of daily protein content from morning milkings

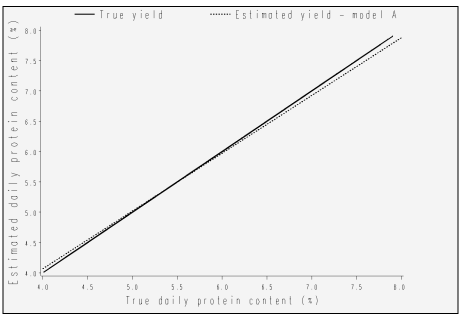
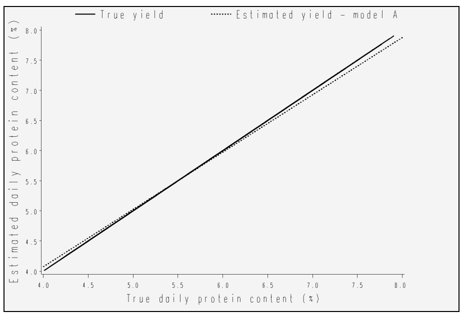


Figure 6

Bias in model A for estimation of daily protein content from evening milkings



The bias in model A for estimation of daily protein content from morning or evening milkings is shown on *Figure 5* and *6*. Lower values of daily protein content were slightly overestimated while the higher values were slightly underestimated, so the bias in model A, both for estimation based on morning or evening milking was negligible.

CONCLUSIONS

Based on present research the following conclusions can be made: the amount of explained variance was slightly lower when estimation of daily milk yield as well as daily protein was based on evening milkings, while the amount of explained variance was slightly higher when daily fat content was estimated from evening milking. With the complexity of the models the amount of explained variance increases and the bias between true and estimated daily yields decreases. Inclusion of other effects in models, beside single milking weights, like effects of lactation stage, breed and number of liveborns, did not significantly increase the amount of explained variance, so the differences between models used for estimation were minor and statistically insignificant, therefore we would recommend use of model A in practice. That model included only partial milk yield as linear regression so, because of its simplicity, the implementation in routine work is simple. Because in present research the information of the interval between successive milkings, which is the most important effect in estimation of daily yields, was not available there is a need for further investigation in which we would be able to take that effect into account.

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Goat milk composition at morning and evening milking

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ABSTRACT

The differences in milk quantity and milk composition between morning and evening milking were studied in a model where the effects of administration of alpha linoleic, eicosapentanoic, and docosahexaenoic fatty acids, individual animal and lactation period were included. The effects of animal and lactation period significantly influenced all variables. Added fatty acids didn't influence fat in milk. The percentage of milk ingredients (fats, proteins, lactose, dry matter without fats and total dry matter) did not differ between morning and evening milking. Average time period between morning and evening milking was thirteen hours and the quantity of milk and milk ingredients was larger at evening, but the production of milk ingredients per time period was faster in the shorter night period.
(Keywords: goats, milk, morning milking, night milking, milk composition)

INTRODUCTION

Differences in milk composition and milk quantity between morning and evening milking are known for longer time. Reduction of costs for production control with only one control per day were the main reason for studying of this phenomena in cows (e.g. *Halgrove et al*., 1984; *Everett et al.,* 1970) and sheep (*Sanna et al*., 1994). *Drobnič et al*. (2000) studied milk quantity and milk composition of goat milk in morning and evening milking from Slovenian herds in production control. The quantity of milk was nearly the same (1.121 g at morning in comparison to 1.106 g at evening milking). No differences in protein and lactose percentage were observed between morning milk (2.98% of proteins and 4.24% of lactose) and evening milk (2.96% of proteins and 4.23% of lactose). Fat concentration in goat milk was much higher in evening (3.50%) than in morning milk (3.13%). Recalculated in absolute production, the total production of fat was 3.62 g larger in daylight period comparing to night period. On the opposite, total production of proteins was 0.67 g and of lactose 0.75 g larger in daylight than in night period. Because the time interval between two controls is unknown, the interpretation of results is difficult. *Simos et al*. (1991) studied milk composition of native Greek goats. The concentration of proteins (3.58% vs. 3.54%) and lactose (4.76 vs. 4.72) at the morning was nearly the same as at the evening. The fat concentration has had other pattern as in the study of *Drobnič et al*. (2000). In the morning, the concentration of fat was 5.30% comparing to 5.06% in the evening.

Different composition of morning and evening milk from morning and evening milking can be interesting for milk consumers. If the differences exist, morning milk might be more suitable for certain use (e.g. fresh use or production of cheese) than the evening milk.

MATERIALS AND METHODS

Sixty-two goats of Saanen and Alpine breed were included in the experiment. The experiment consisted of three time periods. The first, adapting period lasted for 10 days. All animals were treated on same procedure and fed with basic diet. Milk was sampled and analyzed on composition (fat, proteins and lactose) every day. In the second period of the experiment the animals were divided into four groups: first group (EPA) was fed with an extra dosage of 20 g of oil, reach in eicosapentanoic fatty acid (94.93%) and second group (ALPHA) with 20 g of oil per day, reach in alpha linoleic acid (57.84%). Third group (DHA) was fed with 20 g of oil per day, which was reach in docosahexaenoic acid (74.75%). The fourth group was the control group (C), which was fed with basic diet. Basic diet was the same for all four experimental groups. This period lasted for five days. As in the first period, milk was sampled and analyzed every day. Third period lasted for five days. The period started the day after the second period. Animals were fed with standard (basic) diet. Milk was sampled and analyzed every day. Last period started on day fifth after the third period. Animals were treated the same as in period three, but milk quantity was measured only every fifth day nine times until the end of the experiment. At the same time milk was sampled and analyzed.

Basic diet consisted of two kilograms of hay and 700 or 1.000 grams feed mixture with 11.6% ob crude proteins per day. The quantity of feed mixture depended on milk production of the animal. Special vitamin-mineral mixture with 220 g of Ca, 1.5 g of Zn, 88 g of P, 1 g of Fe, 65 g of Na, 1 g of Mn, 3 g of Mg, 20 mg of Cu, 400.000 I.E. of vitamin A, 3 mg of Co, 40.000 I.E. of vitamin D3, 9 mg of I, 1 g of vitamin E, 10 mg of Se per kg was on disposal to animals.

For statistical evaluation of data two statistical models were used. Milk quantity in grams, the quantity of fat (g fat/milking), proteins (g proteins/milking), lactose (g lactose/milking), and dry matter without fat (g DM/milking) and with fat (g TDM/milking) were evaluated with model 1:

$$Y_{ijklm} = \mu + T_i + A_{ij} + M_k + P_l + e_{ijklm},$$

where:

Y_{ijklm} : was the observed trait, μ was mean of the model,

T_i : was treatment (EPA, ALFA, DPA, C),

A_{ij} : was the effect of animal nested within the treatment,

M_k : was the effect of time of milking (morning, evening milking),

P_l : was the experimental period (first or adapting period, second or treatment period, third period and fourth period),

e_{ijklm} : was the individual deviation of every measurement.

The average time difference between morning and evening milking was thirteen and between evening and morning milking was eleven hours. Because it was interesting to estimate the effects of treatment, animal, milking period and experimental period on production rate of observed traits, the quantities of milk and milk ingredients were recalculated in grams produced per hour. Since the exact time of precedent milking for every individual animal was not known, the evening quantity of milk or milk ingredient in milk quantity was divided with eleven and morning quantity was divided with thirteen. The variables are milk production per hour, protein production per hour, fat production per hour, lactose production per hour, DM (dry matter without fats) production per hour and TDM (total dry matter) per hour. The traits were evaluated with model 1.

Observed traits percentage of fat (% fat), percentage of proteins (% proteins), percentage of lactose (% lactose), percentage of dry matter without fats (% DM) and percentage of total dry matter (% TDM) were evaluated with model 2:

$$Y_{ijklm} = \mu + T_i + A_{ij} + M_k + P_l + b\left(x_{ijklm} - \bar{x}\right) + e_{ijklm},$$

where T_i, A_{ij}, M_k, P_l and e_{ijklm} have the same meaning as in the model 1.

Regression coefficient b estimates the effect of milk quantity on percentage of fat, proteins, lactose, DM and TDM in milk. Statistical evaluations were done with procedure GLM (proc GLM) included in statistical package SAS/STAT 8.02.

RESULTS AND DISCUSSION

Results of analysis of variance are shown in *Table 1*. Model 1 explains observed traits relatively good. Coefficients of correlation in model 1 are relatively high. Except for quantity of fat and for production of fat per hour, all the R^2 are higher than 0.7. Individual differences between animals are the most important effect. They influenced all the dependent variables in both statistical models.

Treatment with essential polyunsaturated fatty acids influenced all the observed variables except the total fat quantity in milk and production of fat in grams per time unit. The quantities of milk and milk ingredients like the production of milk ingredients per time unit and percentage of milk ingredients depended on lactation period.

R^2 values in model 2 are much lower than in model 1. That means, that the correction on milk quantity doesn't explain the percentage of milk ingredients as in model 1.

Milking time – morning and evening milking doesn't explain variability in percentage of fat, percentage of DM and TDM. The quantity of milk rather than milking time (morning/evening) explains variability in milk composition. Larger part of variance remains unexplained in model 2.

LSM values for milking period (morning – evening) are shown in *Table 2*. As expected, the larger quantity of milk was earned at evening milking (787 vs. 682 g), where the period between two milking was for two hours longer than between evening and morning milking. The same can be concluded for quantities of fat, proteins, lactose, DM and TDM. Total production of fat was in daylight period for 13% higher than in night period. The differences in total protein, lactose, DM and TDM production were in daylight period still larger – around 15% than production of fat. On the other hand, the period between morning and evening milking was on average 18% longer than the period between evening and morning milking. Production of milk ingredients, expressed as production of milk ingredient in grams per hour shows faster production of all studied components. All differences between the groups were statistically significant according to model 1. There were no proven differences in percentage of fat, DM and TDM between morning and evening milking, but statistically significant larger percentage of proteins and lactose was observed in morning than in evening milk. All regression coefficients were positive and different from zero. Larger amount of milk doesn't mean lower concentration of milk ingredients. In this case larger milk amount rather means higher concentration of fat, proteins, lactose, DM and TDM.

Table 1

Results of analysis of variance according to model 1 and model 2

			model				T_i		A_{ij}		M_k		P_l		$b(x_{ijklm}-x)$	
			df	F.	P	R^2	df	P	df	P	df	P	df	P	df	P
1	model 1	milk quantity	65	171.18	<0.0001	0.7945	3	<0.0001	58	<0.0001	1	<0.0001	3	<0.0001		
2		g fat/milking	65	89.18	<0.0001	0.6682	3	0.1588	58	<0.0001	1	<0.0001	3	<0.0001		
3		g proteins/milking	65	125.72	<0.0001	0.7395	3	0.0036	58	<0.0001	1	<0.0001	3	<0.0001		
4		g lactose/milking	65	150.27	<0.0001	0.7724	3	<0.0001	58	<0.0001	1	<0.0001	3	<0.0001		
5		g DM/milking	65	141.88	<0.0001	0.7622	3	<0.0001	58	<0.0001	1	<0.0001	3	<0.0001		
6		g TDM/milking	65	134.39	<0.0001	0.7522	3	<0.0001	58	<0.0001	1	<0.0001	3	<0.0001		
7		g milk/hour	65	170.70	<0.0001	0.7940	3	<0.0001	58	<0.0001	1	<0.0001	3	<0.0001		
8		g fat/hour	65	87.78	<0.0001	0.6647	3	0.1563	58	<0.0001	1	<0.0001	3	<0.0001		
9		g proteins/hour	65	123.77	<0.0001	0.7365	3	0.0026	58	<0.0001	1	0.0001	3	<0.0001		
10		g lactose/hour	65	148.53	<0.0001	0.7704	3	<0.0001	58	<0.0001	1	0.0014	3	<0.0001		
11		g DM/hour	65	140.67	<0.0001	0.7606	3	<0.0001	58	<0.0001	1	0.0002	3	<0.0001		
12		g TDM/hour	65	132.93	<0.0001	0.7501	3	<0.0001	58	<0.0001	1	<0.0001	3	<0.0001		
13	model 2	% fat	66	34.00	<0.0001	0.4396	3	<0.0001	58	<0.0001	1	0.6222	3	<0.0001	1	<0.0001
14		% proteins	66	76.54	<0.0001	0.6384	3	<0.0001	58	<0.0001	1	0.0016	3	<0.0001	1	<0.0001
15		% lactose	66	35.03	<0.0001	0.4469	3	<0.0001	58	<0.0001	1	0.0367	3	<0.0001	1	<0.0001
16		% DM	66	105.64	<0.0001	0.7093	3	<0.0001	58	<0.0001	1	0.0793	3	<0.0001	1	<0.0001
17		% TDM	66	82.46	<0.0001	0.6558	3	<0.0001	58	<0.0001	1	0.5566	3	<0.0001	1	<0.0001

Table 2

LSM values for morning and evening milking according to model 1 and model 2 and regression coefficients for effect of milk quantity according to model 2

	model	trait	LSM			regression	
			morning	evening	P	b	P
1	1	milk quantity	682.42	786.84	<0.0001		
2		g fat/milking	19.94	22.49	<0.0001		
3		g proteins/milking	20.98	24.16	<0.0001		
4		g lactose/milking	30.52	35.33	<0.0001		
5		g DM/milking	56.94	65.61	<0.0001		
6		g TDM/milking	76.88	88.06	<0.0001		
7		g milk/hour	62.20	60.32	<0.0001		
8		g fat/hour	1.82	1.72	<0.0001		
9		g proteins/hour	1.91	1.85	0.0001		
10		g lactose/hour	2.78	2.71	0.0014		
11		g DM/hour	5.19	5.03	0.0002		
12		g TDM/hour	7.01	6.75	<0.0001		
13	2	% fat	2.98	2.97	0.6222	0.000425	<0.0001
14		% proteins	3.17	3.21	0.0016	0.000556	<0.0001
15		% lactose	4.51	4.49	0.0367	0.000245	<0.0001
16		% DM	8.48	8.50	0.0793	0.000310	<0.0001
17		% TDM	11.45	11.47	0.5566	0.000735	<0.0001

It can be concluded, that the night production of milk and milk ingredients was faster than the daylight production. Because of unequal time periods between morning and evening and between evening and morning milking the differences in milk composition might not be correctly expressed, since the dynamics of production of milk and milk ingredients is not known within those time periods. The difference cannot be only the consequence of resting of animals during the night period, but it is also the consequence of longer time period as such. No benefits of morning or evening milk were found.

CONCLUSIONS

In the experiment with 62 goats of Saanen and Alpine breed the effect of individual animal, the effect of longtime administration of EPA, ALFA and DHA fatty acids, the effect of lactation period and the effect of morning/evening milking on milk quantity, on milk ingredients production and milk composition was studied with two statistical models. The quantities of milk, milk ingredients and production of milk ingredients per time unit were explained with very high R^2. On the other side, R^2 values in models, where milk ingredients were expressed in percentage, were much lower. The effects of animal and time period influenced all the dependent variables. Administration of special fatty acids didn't influence fat quantities or percentage in milk and the explanation of variability in milk ingredients was low where they were expressed in percentages. The quantities of milk and milk ingredients were generally higher at evening milking, but the production of milk and milk ingredients per time unit was faster in the shorter night period.

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SECTION 4

POULTRY BREEDING

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The effects of free range keeping and cage system on the plumage status of Prelux-G laying hens

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ABSTRACT

Plumage damage was measured on hens of Slovenian provenance Prelux-G in two housing systems. Hens were grown from one-day old as a single group on deep litter. At 18 weeks of age, they were randomly assigned to group of 113 hens in a conventional cage system (group I) and to a group of 50 hens and one cock in a free range system (group II). Plumage observations were performed individually at the age of 36 and 72 weeks. The body of a layer was divided into six parts that were measured for denuded areas. Two parts (wings-primary feathers and tail) were examined for damaged and broken feathers. Significantly worse plumage status was found on the head and neck above, head and neck below, breast and wings-coverts at both recordings (36 and 72 weeks) in the group I compared to group II. In contrast, group I had a significantly better feather status on the back compared to group II that is ascribed to the presence of a cock in group II. Denuded areas significantly increased with age for head and neck above in group I, for head and neck below in both groups and for back also in both groups. At the age of 72 weeks more damage for wing primaries was recorded in group I, while more damage for tail feathers was noticed in group II.
(Keywords: hens, plumage status, free range, battery cages)

INTRODUCTION

Consumers are increasingly interested in the safety and origin of their food and the ethical issues within the production chain. Even though organic poultry are given possibility to species-specific behaviour by allowing access to outdoors and rearing them in free range-systems, organic and other free-range systems present both positive and negative welfare consequences to chickens, as compared to caged and confined systems. For example, feather pecking in laying hens occurs both in conventional battery cages and in alternative housing systems (*Appleby* and *Hughes*, 1991). It causes animal welfare problems, as it may lead to injuries, economic losses because of increased food consumption in deplumed birds and even the death of birds (*Mahboub*, 2004). The status of birds' integument has a considerable impact on the interpretation of their health and welfare. This applies also in evaluation of different housing systems in commercial production. Most studies of laying hen welfare in different housing systems therefore now include an assessment of integument condition (*Tauson et al.*, 2005). The present research was part of larger project whose main goal was to get a knowledge about suitability of Prelux-G laying hens to perform under the rules of confined (cages) and organic (free range) production. The aim of this research was to record the welfare status of Prelux-G hens by measuring their plumage status in two housing systems: organic (free range) and conventional battery cages.

MATERIALS AND METHODS

Hundred and sixty-three laying hens of Slovenian provenance Prelux-G were included in the study and reared according to standard deep litter technology till 18 weeks of age. Birds were not beak-trimmed. At 18 weeks of age 113 hens were moved into individual cages (floor area of 1250 cm^2/cage) in the three-floor batteries in the hen-house without windows, while 50 pullets and one cock were placed in the free range. Hens in free range were housed in a brick house. There were 1.8 hens per m^2 in the house on straw and shavings. The brick house was equipped with a sand bath (0.2 m^2 trough with silicious sand), eight individual nests, round drinker, two round feeders, a hen-perch, and a window surface 1.9 m^2. Additional light was provided in free range after 20 weeks of age. The light in battery house was increased gradually until 14 h light: 10 h dark at 31 weeks of age. The free range area varied during the rearing period between 4 m^2 and 12 m^2 per hen. Free range areas were available from the moving-in till the end of rearing. The shortest free range was used in December (6 hours a day) and the longest in summer months (12 hours a day). Since the aim of the study was to compare plumage status of Prelux-G laying hens under Slovenian production conditions the standard feeding mixture for hens in battery cages and certified organic poultry diet for hens in free range were used. Hens in battery cages were fed *ad libitum* on the complete feeding mixture containing 17.0% crude protein and 3.0% crude fat. In free range hens were fed on organic feeding mixture "Biokraft Lege" (Unser Lagerhaus Warenhandels Gmbh, Klagenfurt) with daily controlled amounts. Organization which certify organic poultry and eggs in Slovenia requires that most of the ingredients in poultry rations be organically grown. Because of that we *checked whether a diet is acceptable to organic certifying organization and additionally we analysed the chemical composition of organic feeding mixture which contained 16.0% crude protein and 3.2% crude fat.* Hens finished all feeds. The farmer also strewed 1 kg of oats on litter and depending on the time of the year the hens were able to find a part of their feed by scavenging in the pasture. Feed intake on the pasture was not recorded. All birds were marked with metal leg rings. Hen weight, egg production, feed consumption, mortality and plumage status were recorded. In *Table 1*, the production results as affected by housing system for the Prelux-G laying hens are summarized.

Table 1

Production results by housing system for prelux-G laying hens

Group	Cumulative number of eggs per hen-housed	Hen-housed egg production (%)	Mortality (%)	Feed g/day/hen	Feed g/egg	Body weight at 72 wk of age (kg)
I (cages)	297	80.8	3.5	120.3	147.6	2.5
II (free range)	282	77.5	2.0	135.4*	178.1*	2.4

*without feed found on the pasture.

Hen-housed egg production for a production period of 52 weeks was worked using the following formula: 100×total number of eggs produced by a flock/364×total number of hens housed. Cage egg production extended from 17.5% (21 weeks) to 94.4% (33 weeks) with a mean of 80.8%, and similar values for free range egg production ranged from 9.1% (21 weeks) to 88.8% (35 weeks) with a mean of 77.5% (*Table 1*). The difference between two types of housing systems was only 3.3% of eggs for a period of 52 weeks. Cumulative egg production was 297 eggs/hen-housed in cages and 282 eggs/hen-housed in free range (*Table 1*). Mortality rate during 52 weeks of production period was higher in cages (3.5%) than in free range (2.0%) The feed required per one egg showed better results for the cages (147.6 g/egg) than for the free range (178.1 g/egg, *Table 1*). For scoring of hen' integument several methods of scoring systems (subjective scoring, planimetry) have been presented during the years.

Since using a total body score can only hardly explain or describe possible reasons for the deterioration of the plumage (*Freire et al*., 1999; *Kjaer*, 2000) or wear from different parts of the environment (*Tauson*, 1984) we used planimetry for measuring denuded areas on six body parts (head, neck above; head, neck below; back; breast; abdomen; wings-coverts). Based on the estimation scheme (*Figure 1*) we first measured with a tape measure and later on calculated the surface of denuded and frayed areas. Flight feathers (wings-primary feathers; tail) were differentiated in number and also in damaged compared to feathers from the rest of the body.

Figure 1

The body parts used for measuring plumage status: 1 - head, neck above; 2 - head, neck below; 3 - back; 4 - breast (breast-bone area); 5 - abdomen (back part); 6 - wings (6a – coverts, 6b – wing primary feathers); 7 - tail (adapted from *Keppler et al*., 2001)

Due to the nature of distributions of denuded areas the studied traits were analysed with the procedure GENMOD with generalized linear models. Housing system and age of hens as fixed effects with levels and interaction between them were included into the statistical model. Log-normal distribution was assumed. Since many measurements had value 0 before log calculations, a small positive value (0.01) was added. Damages to the wing primary feathers and tail feathers were not included into statistical analyses because they were not measured but only noticed in the case of presence. Model used was $y_{ijk}= \mu + R_i + S_j + RS_{ij} + e_{ijk}$ where y_{ijk} was assessment of denuded areas on the certain part of hen body; μ was population mean value; R_i was fixed effect of i-th housing system; S_j was fixed effect of j-th age of hens; RS_{ij} was interaction between housing system and age of hens; e_{ijk} was residual.

RESULTS AND DISCUSSION

In both housing systems the most widely extended denuded areas were found on abdomen and breast and the lowest on wings-coverts and back. This is in accordance with *Mahboub* (2004) who at the age of 35 weeks recorded large denuded areas on the breast among Lohmann Traditional hens housed in three systems (poultry house (floor system) without grassland − group I; poultry house (floor system) with 2.5 m^2 grass area/hen – group II; poultry house (floor system) with 10 m^2 grass area/hen – group III). Housing system does not significantly affect the plumage status on abdomen (p=0.5626) while for all other parts of the body a significant difference (p<0.0001) was noticed between housing systems (*Table 2*). The age of hens had a statistically significant influence on estimated plumage status in all parts of birds (p<0.0001). This is in accordance with what was reported in the literature that regardless of used housing systems, feather cover of laying hens usually deteriorates by age (*LaBrash* and *Scheideler*, 2005). The causes of plumage deterioration are mainly two: feather pecking and/or abrasion against equipment. *Bilcik* and *Keeling* (1999) suggested that measuring denuded areas is considered a reliable method for the assessment of feather pecking activity in the flock.

Table 2

Sources of variability and statistical significance of their influence on plumage status in certain parts of birds and estimations of differences between housing system and age (estimated differences are on log scale)

Body part	p-value			Estimated difference ± SE	
	Housing system	Age	Housing system×Age	Housing system	Age
Head and neck above	<0.0001	<0.0001	<0.0001	6.97±0.22	-1.32±0.22
Head and neck below	<0.0001	<0.0001	<0.0001	5.02±0.24	-3.94±0.24
Back	<0.0001	<0.0001	<0.0001	-0.98±0.24	-5.34±0.24
Breast	0.0002	<0.0001	0.2140	1.05±0.27	-2.40±0.27
Abdomen	0.5626	<0.0001	0.1798	-0.14±0.25	-1.64±0.25
Wings-coverts	<0.0001	<0.0001	0.1542	3.22±0.29	-2.78±0.29

SE – standard error.

On the other hand, *Kjaer* (2000) explained that the lack of correlation between feather pecking behaviour and plumage condition might be attributed to the fact that a high intensity of feather pecking soon results in a denudation of most hens, after which point further feather pecking only has a little damaging effect and therefore cannot be documented by scoring of the plumage condition. Scores for plumage condition may be also very useful for explaining causes to increases in energy requirement from poor insulation of the body. In one of such studies *Ward et al.* (2001) discovered that due to the condition of the feathering, in the pectoral region, plumage of free range birds was more resistant to heat loss from the body than that of broiler birds.

The interaction between housing system and age was statistically significant for head and neck above, head and neck below and for back (*Table 2*). Since the interaction between housing system and age had a statistically significant influence on head and neck above and below as well as on back for these parts of bodies the estimated mean values were exposed.

In head and neck above of hens in battery cages at age 36 and 72 weeks a significant augment of denuded areas was noticed (between 2.86 cm^2 and 39.65 cm^2). In both ages the difference between housing systems referring to the denuded areas were statistically significant ($p<0.0001$), with exception of free range hens at age 36 and 72 weeks ($p=1.0000$).

Concerning the head and neck below the interactions between ages and housing systems were always statistically significant. The antilog of estimations of mean values found significantly increased values at higher age of hens. Therefore denuded areas in battery cages augment with bird age from 10.28 cm^2 to 77.48 cm^2. In free range the denuded areas are smaller but the difference among ages is high (between 0.01 cm^2 and 5.53 cm^2).

Regarding the denuded areas on the back only at age 36 weeks no statistically significant differences among housing systems ($p=1.0000$) was found while in all other cases the influence of housing system in interaction with bird's age was statistically significant ($p<0.0001$). Except head and neck below and neck above the free range hens had more extensive denuded areas on the back during laying period in comparison to the hens from battery cages.

Planimetric measurements of denuded areas on head and neck above and below, back, breast, abdomen and wings-coverts were statistically analysed. We used the method with log measured values. In battery cages the estimations of mean value of back (-2.43) and head and neck below (3.34) (*Table 3*) deviated most. Antilog to the results in Table 3 showed the estimated mean value in cm^2. In back it was 0.18 cm^2 and in head and neck below 28.22 cm^2. In free range the estimated mean value was the highest in abdomen (11.94 cm^2) and the lowest in the head and neck above (0.01 cm^2).

At age 36 and 72 weeks the most frayed and denuded were abdominal areas (4.90 cm^2; 25.28 cm^2) following by head and neck below at age 72 weeks (16.45 cm^2) and breast (18.54 cm^2). *Mahboub* (2004) observed that in free range feather pecking on the belly was the most pronounced followed by rump and tail. From these findings, the belly area was the main region pecked in the hens that spent more time in the outside winter garden. In this run, the target bird spent more time in dustbathing, foraging and scratching and thereby facilitates access to the belly in these positions.

In the battery cages in most hens we found damages to the wing primary feathers at age 72 weeks following by hens with damages to the tail feathers. In free range at age 72 weeks there were nearly half of hens with damages to the wing primary feathers while the percentage of hens with damages to the tail feathers was significantly higher. The damage on the primary wing feathers and tail may be attributed to abrasion at the walls of the passages between inside and outside areas.

Probably due to the abrasions on the cage front was the feather loss on head and neck below more pronounced in the cages than in the free range. According to *Hughes* (1980) feather loss from abrasion is typically worse in cages.

Hens in a free range exhibited significantlly larger denuded areas on backs in comparison with caged hens. This observation may be explained by the presence of mature cock and mating process. The relationship between the presence of cockerels with the hens and feather pecking is unclear. *Odén et al.* (1999) described positive effect of cocks in the flock on reduction of aggressivity, but no effect on feather pecking behaviour. Contrarily, *Bestman* and *Wagenaar* (2003) found the presence of cockerels in the flock of hens to be a factor preventing feather pecking.

Table 3

Least square means (LSM ± SE) for denuded areas in certain plumage areas of hens (LSM are on log scale)

Body part	LSM ± SE			
	Housing system		Age (weeks)	
	Battery cages	Free range	36	72
Head and neck above	2.36±0.15	-4.61±0.16	-1.78±0.15	-0.46±0.16
Head and neck below	3.34±0.17	-1.68±0.18	-1.14±0.17	2.80±0.18
Back	-2.43±0.17	-1.45±0.17	-4.61±0.17	0.73±0.17
Breast	2.24±0.19	1.19±0.19	0.52±0.19	2.92±0.19
Abdomen	2.34±0.17	2.48±0.18	1.59±0.17	3.23±0.18
Wings-coverts	0.21±0.20	-3.01±0.21	-2.79±0.20	-0.01±0.21

LSM – least square mean, SE – standard error.

CONCLUSIONS

The free range hens, in comparison to the caged birds, had significantly better feather condition on head and neck above, head and neck below, breast, wings-coverts and poorer feather condition on back. Caged hens experienced severe feather loss on head and neck below as they rubbed constantly against the wire cages. In free range as well as in battery cages the most extensive denuded areas were found in the abdominal part.

The highest percentage of hens in both housing systems had damages to the wing primary feathers following by tail feathers while damages to the cover feathers were hardly noticed. In battery cages we noticed more frayed feathers and damages to the feathers and skin.

Beside plumage condition welfare of animals can be also assessed by registering mortality. The mortality was higher in the battery cages than in the free range. Claims that conversion to cage-free housing would necessarily increase mortality are thus not supported by present scientific data.

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Effect of different phosphorus intakes on phosphorus balance and performance of layers during peak production

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ABSTRACT

Two experiments were conducted to determine the effect of different phosphorus supplies on phosphorus balance and performance of high performance layers. Both experiments were conducted with 72 HyLine layers (18 birds/treatment; 3 birds/cage). Hens were fed diets containing 2.5; 2.0; 1.5 and 1.0 g/kg non-phytate phosphorus (NPP). The calcium content was maintained constant at 32.5 g/kg. In the balance trial, the hens were 45 weeks of age (25 week of production). At the beginning of the performance trial the birds were 45 weeks old and were fed for 12 weeks. Absolute P retention was calculated subtracting the mean P excretion in the excreta from the P intake. Relative P retention was calculated absolute P retention dividing by P intake. Decreasing dietary NPP levels reduced P excretion in the excreta. Relative P retention was not influenced by levels of NPP. Decreasing dietary NPP reduced absolute P retention and P balance. Hens fed 1.5 and 1.0 g/kg NPP showed the lowest P balance at 43 and 39 mg/d, respectively, which was still adequate for the birds. Diets had no effect on feed intake, egg weight and feed per egg mass ($P \geq 0.05$). As NPP decreased from 1.5 to 1.0 g/kg, egg production and egg mass decreased ($P \leq 0.05$). It was concluded that dietary P content can be reduced up to 1.5 g/kg NPP without influencing the level of production, and at the same time the environment is also alleviated.
(Keywords: dietary phosphorus, phosphorus balance, phosphorus retention, egg production)

INTRODUCTION

Calcium and phosphorus are critical nutrients for ensuring maximum egg production and good eggshell quality. The calcium and phosphorus requirements for hens appear to be changing continuously. non-phytate phosphorus (NPP) requirements for laying hens have constantly declined during the last 40 years, in part due to increasing concern about environmental issues and pollution, which in certain countries led to strict legal limitations of P excretion. A number of researchers have reported that dietary phosphorus can be reduced without an adverse effect on performance (*Usayran* and *Balnave*, 1994; *Castillo et al.,* 2004; *Kovacs et al.*, 2006). Decreasing total phosphorus can reduce faecal phosphorus excretion, up to an estimated 20%, which is a significant goal to strive for in reducing potential phosphorus pollution problems (*Summers*, 1995). Results of *Scheideler* and *Jerry* (1986) showed that lower dietary phosphorus levels decreased absolute phosphorus retention, however, this finding is not consistent with the results of *Keshavarz* (1985), who found that while decreasing dietary phosphorus had no effect on absolute retention, it did increase the percentage retention. Egg shell quality is of major importance to the egg industry worldwide. A number of investigators have reported that low levels of phosphorus improved shell quality (*Usayran* and *Balnave*, 1994). The last *NRC* (1994) recommendation is 2.5 g/kg NPP. This may be in excess of the current requirements of the laying hen.

The effects of reduced dietary phosphorus levels still need to be investigated. The following experiments were conducted to determine the effect of different phosphorus supplies on phosphorus balance and performance of high performance layers during peak production.

MATERIALS AND METHODS

Balance trial

The experiment was conducted with a total of 72 HyLine layers (18 birds/treatment; 3 birds/cage) 45 weeks of age (25 weeks of production). Feed and water were provided *ad libitum* during the experiment. From the start of laying at 20 weeks to 45 weeks of age, the hens received the same diets as in the experiment. The diets contained 2.5; 2.0; 1.5 and 1.0 g/kg NPP. The calcium content was maintained constant at 32.5 g/kg (*NRC*, 1994). The levels of energy, crude protein, lysine and methionine were kept constant in all diets. Composition and nutrient content of diet ingredients are shown in *Tables 1*.

Table 1

Composition and nutrient contents of the experimental diets (g/kg)

Ingredient	Treatments			
	1	2	3	4
Corn	655.0	655.0	655.0	655.0
Soybean meal (CP: 47.3%)	214.0	214.0	214.0	214.0
Salt	31.6	33.0	34.5	36.1
Limestone	78.7	79.7	80.6	81.5
MCP	8.1	5.8	3.4	0.8
NaCl	3.7	3.7	3.7	3.7
Premix[1]	5.0	5.0	5.0	5.0
Lizin-HCl	1.3	1.2	1.2	1.3
DL-metionin	2.6	2.6	2.6	2.6
Total	**1000.0**	**1000.0**	**1000.0**	**1000.0**
Nutrient content				
AMEn (MJ/kg)[2]	11.4	11.4	11.4	11.4
Crude protein	163.1	163.0	163.0	163.1
Crude fat	24.0	24.0	24.0	24.0
Lysine[2]	8.8	8.8	8.8	8.8
Met + Cys[2]	7.8	7.8	7.8	7.8
Ca	**32.5**	**32.5**	**32.5**	**32.5**
Total P	4.9	4.3	3.8	3.2
Non-phytate P[2]	**2.5**	**2.0**	**1.5**	**1.0**

[1]Provided per kilogram: calcium 186 g; iron 20000 mg; zinc 15000 mg; iodine 90 mg; manganese 20000 mg; selenium 60 mg; copper 1600 mg; vitamin A 1935600 NE; vitamin D_3 387200 NE; vitamin E 3880 NE; vitamin K_3 388 mg; vitamin B_1 388 mg; vitamin B_2 1280 mg; vitamin B_6 520 mg; vitamin B_{12} 2.8 mg; calcium pantothenate 3200 mg; niacin 9040 mg; folic acid128 mg; biotin 28.5 mg; choline 59983 mg.
[2] Calculated values.

Total amount of excreta was collected and measured for 4 days. Feed consumption and daily egg production were recorded throughout the collection period. All eggs were individually weighed. At the beginning and end of collection period body weights were individually recorded.

Performance trial

The experiment was conducted with a total of 72 HyLine layers (18 birds/treatment; 3 birds/cage). Feed and water were provided *ad libitum* during the experiment. The trial started at 45 weeks of age (week 25 of production) and lasted for 12 weeks. From 20 to 45 weeks of age, the hens were fed the same diets as in the balance trial. Egg production was recorded daily. All eggs were collected and individually weighed. Feed consumption was determined weekly. Individual body weight and shell strength and thickness were measured every 4^{th} week.

Chemical Analyses

The nutrient content of diets and dry matter for both balance and performance trials, calcium and phosphorus content of excreta and eggs were determined in according to *AOAC* (1999).

Calculations

Absolute P retention was calculated subtracting the mean P excretion in the excreta from the P intake. Relative P retention was calculated absolute P retention dividing by P intake.

Statistical analysis

Statistical analyses were carried out by variance analysis (*SAS*, 2001) for both balance and performance trials according to the following general model:

$$Y_{i,j}=\mu+A_i+e_i$$

Where: μ= overall mean; A_i= phosphorus levels (i=1,2,3,4); $e_{i,j}$= residual error.

In case of a significant treatment effect, mean differences were tested by Tukey test, at P=0.05 level.

RESULTS AND DISCUSSION

Data of the phosphorus balance and performance trials are shown in *Tables 2* and *3*.

Balance trial

Decreasing the dietary NPP had no effect on feed intake therefore the significant change in P intake is attributable to the different dietary P levels ($P\leq0.05$). At the NPP levels used, phosphorus excretion in the excreta were 341, 324, 285 and 242 mg/d respectively. A 40% (Treatment 3) and 60% (Treatment 4) reduction of the NPP level resulted in an approximately 16% and 30% reduction, respectively of P excretion in the excreta ($P\leq0.05$). This finding appears to agree with those of *Summers* (1995). As the dietary NPP decreased (from 2.5 to 1.0), absolute P retention decreased (from 197 to 130 mg/d) ($P\leq0.05$) but there was no significant effect on percentage retention (34.7% on average) ($P\geq0.05$). This finding was consistent with the results of *Scheideler* and *Jerry* (1986) but does not correspond with the results of *Keshavarz* (1986) who found on the contrary, that changing the dietary levels of phosphorus did not influence phosphorus retention in absolute terms, but had an effect on percentage phosphorus retention. Phosphorus excretion in the egg was not influenced by treatments in hens fed 2.5; 2.0 and 1.5 g/kg NPP ($P \geq 0.05$), however, it was lower in hens fed 1.0 g/kg NPP because the egg production of those hens decreased. Decreasing NPP from 2.5 to 2.0 and 1.5 g/kg, respectively decreased the phosphorus balance from 94 to 66 and 43 mg/d, respectively (both reductions were significant at $P \leq 0.05$). The further reduction of dietary NPP did not decrease the phosphorus balance any further.

Table 2

Influence of dietary non-phytate phosphorus on the results of the balance (mg/d)

Items	Treatments				
	1	**2**	**3**	**4**	**RMSE***
P intake	537^{a}	485^{b}	430^{c}	371^{d}	13
P excretion in the excreta	341^{a}	324^{a}	285^{b}	242^{c}	15
P retention	197^{a}	161^{b}	145^{cb}	130^{c}	11
P retention (%)	36.6^{a}	33.3^{a}	33.7^{a}	35.0^{a}	2.3
P excretion in the egg	102^{a}	95^{ab}	102^{a}	90^{b}	6
P balance	94^{a}	66^{b}	43^{c}	39^{c}	10

* : Root mean square error.
$^{a\text{-}d}$: Means in each row without common superscript differ ($P \leq 0.05$).

Performance trial

Feed consumption was not influenced by treatments ($P \geq 0.05$; *Table 2*). This finding was not consistent with the results of *Sohail* and *Roland* (2002), where feed consumption was lower when 1.0 g/kg NPP was fed. Average egg production did not change (95%) in layers fed 2.5; 2.0 and 1.5 g/kg NPP. *Usayran* and *Balnave* (1994) and *Summers* (1995) found the same.

Table 3

Influence of dietary non-phytate phosphorus on the results of the performance trial

Items	Treatments				
	1	**2**	**3**	**4**	**RMSE***
Feed intake (g/d)	120^{a}	117^{a}	120^{a}	113^{a}	7
Egg production (%)	95.9^{a}	94.6^{a}	94.7^{a}	86.9^{b}	4.0
Egg weight (g/egg)	64.2^{a}	63.2^{a}	64.8^{a}	62.9^{a}	2.0
Egg mass (g/d)	61.5^{a}	59.8^{a}	61.3^{a}	54.7^{b}	3.6
Feed per egg mass (kg/kg)	1.93^{a}	1.96^{a}	1.95^{a}	2.07^{a}	0.10
Shell strength (kg/egg)	2.96^{a}	2.74^{a}	2.80^{a}	2.88^{a}	0.86
Shell thickness (mm)	0.333^{a}	0.325^{a}	0.325^{a}	0.323^{a}	0.030

* : Root mean square error.
$^{a,\,b}$: Means in each row without common superscript differ ($P \leq 0.05$).

Leeson et al. (1993), however, found that when the total phosphorus levels were reduced from 5.8 to 5.1 g/kg egg production increased, these total P levels, however, were considerably higher than the levels fed in our trial. As NPP decreased from 1.5 to 1.0 g/kg, egg production decreased ($P \leq 0.05$), this result is consistent with the findings of *Sohail* and *Roland* (2002). Egg weight was not significantly affected by dietary levels of NPP ($P \geq 0.05$) in contrast to *Sohail* and *Roland* (2002). They found that reducing NPP from 3.0 to 1.0 g/kg decreased egg weight. Egg mass showed the same tendency as egg production, with a reduction when hens were fed 1.0 g/kg NPP ($P \leq 0.05$). On average, 1.98 kg of feed was used for producing 1 kg of egg mass. Diets had no effect on egg shell strength and thickness ($P \geq 0.05$).

CONCLUSION

It can be concluded from the balance trial that the decreasing dietary NPP levels reduce P excretion in the excreta. Decreasing the dietary NPP reduced absolute P retention and P balance. Hens fed 1.5 and 1.0 g/kg NPP showed the lowest P balance at 43 and 39 mg/d, respectively, which was still adequate for the birds. Results of the performance trial show that reduction of NPP from 2.5 to 1.5 g/kg had no effect on egg production, a further reduction to 1.0 g/kg, however, decreased egg production.

Dietary P content can be reduced up to 1.5 g/kg NPP without influencing the level of production, and at the same time the the environment is also alleviated. Therefore we suggest to feed layers in peak production with diets containing 1.5 g/kg NPP.

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Commercial quality evaluation of different weight grade eggs

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ABSTRACT

In the Republic of Croatia, consumers are offered eggs of extra quality class, and of I and II quality classes, all in different weight grades. Prices of eggs are primarily formed on the basis of market supplies and demands, and secondly by the egg weight. This research was carried out with the aim to evaluate market quality of S, A and B weight grade eggs at our market. The obtained research results will be used to inform consumers on the quality of commercial eggs. Analyzed eggs (n=150) were bought at a family-owned poultry farm. In order to assess quality of eggs, the following were investigated: egg weight, portions of main parts in egg, shell thickness and firmness, albumen height, yolk color, pH of yolk and albumen, HU (Haugh units) and VN (value number) of eggs. It was determined that eggs of the S weight grade had more intensive color of yolk (13.08) than eggs of the A and B weight grades (12.83 and 12.76, respectively), P<0.05. Other investigated traits (portions of main parts in egg, shell thickness and firmness, albumen height, pH of yolk and albumen, HU and VN of eggs) proved no statistically significant differences (P>0.05). Furthermore, there was a percentage of protein and dry matter determined in eggs of different weight grades, on the basis of which the relation between price and quality was postulated.
(Keywords: eggs, market quality, freshness, price, weight grade)

INTRODUCTION

Eggs are an excellent source of protein, vitamins, minerals and high quality fat, such as phospholipids and unsaturated fatty acids (PUFA) (*Seuss-Baum*, 2005). Through feeding regimes it is almost impossible to influence changes in content of total protein and amino acids in eggs. However, through supplementation of different forages in diets fed to laying hens, it is relatively easy to manipulate contents of lipids, fatty acids (*Meluzzi et al.,* 2000; *Kralik et al*., 2005), fat soluble vitamins (*Flachowsky et al*., 2000) and minerals (*Surai*, 2002; *Yaroshenko et al*., 2003). Depending on its weight, an egg provides 4.5–6 g of protein, with the half of that amount being contained in albumen. It also contains 65–75 kcal and 185–215 mg of cholesterol in yolk. If referring to their price, eggs are a cheap foodstuff. When buying eggs, customers often dwell on whether to buy domestic free-range eggs or eggs produced on farms, or eggs that weigh more or less. Science cannot provide a simple answer to these questions, as the purchase of eggs is a matter of customers' personal choice. In the last 5 years, Croatia marks an increase in egg production (*Table 1*). The table shows that only 33% of commercial eggs were produced by industrial farms, and even 67% by family-owned farms. In 2004, there were 801 mil. of eggs produced, out of which 257 mil. on industrial farms and 544 mil. on family-owned farms. If considering yearly consumption of eggs and powder eggs per capita, in the last 5 years some fluctuations were noticed. The highest consumption was marked in 2001

(10.81 kg), while in 2004 it reached only 8.15 kg. Decrease in eggs and powder eggs consumption can be explained by the world-wide occurrence of Avian influenza, and by consumers' awareness on high content of cholesterol in egg yolk and its negative effects on human health (*Maluzzi, et al*., 1995). In the near future, increase of fresh egg consumption will be possible to achieve through production of „functional food", i.e. designed eggs that will significantly differ in their content from currently available commercial eggs.

Table 1

Production and consumption of eggs in Croatia

Year	Egg production (mil. pcs)			Eggs and powder eggs consumption (kg per capita)
	Business entities and industrial farms	Family-owned farms	Total	
2000	272	502	774	9.32
2001	287	500	787	10.81
2002	274	487	761	10.72
2003	292	581	873	8.12
2004	257	544	801	8.15

Source: Croatian Statistical Yearbook, 2005.

According to the Regulations on eggs' and egg products' quality (Croatian Official Journal No. 55/96), in Croatia commercial chicken eggs are divided into 10 weight grades: E less than 45 g; D 45–50 g; C 50–55 g; B 55–60 g; A 60–65 g; S 65–70 g; SU 70–75 g; SV 75–80 g; SZ 80–85 g and SX 85 g and more. Commercial eggs are further divided into 4 quality classes (extra class, I, II and III quality class). It is important to emphasize that eggs of the III quality class are used only in further processing. Differently from our market, European Union market offers since 2003 eggs that are classified into 4 weight grades: S less than 53 g; M 53–63 g; L 63–73 g and XL more than 73 g. Market quality of eggs is determined on the basis of several traits referring to their appearance and inner freshness. The market demands eggs that have clean, firm, unbroken and unwashed shell. Of their inner traits, commercial eggs must have clear and compact albumen, satisfactory color of yolk, HU of at least 70, they should be odor-free and have the air container below 9 mm. As the egg size significantly affects market price of eggs, this research was carried out with the aim to assess quality of eggs in order to provide relevant information to consumers and make their choice in purchasing eggs easier.

MATERIALS AND METHODS

The research focused on eggs of extra quality in different weight grades (S, A, B), all bought on a family-owned farm. A total of 150 eggs were analyzed, of which 50 were of S grade, 70 of A grade and 30 of B grade. Eggs were prepared for marketing (classified and appropriately packaged).

In order to assess the quality of eggs, the following traits were evaluated: weight of eggs and their main parts (g), portions of main parts in egg (%), thickness (mm) and firmness of egg shell (kg/cm^2), albumen height (mm), yolk color, pH of albumen and yolk, HU and VN of eggs. Physical traits of eggs were determined in Egg Multi-Tester EMT 5200 and Eggshell Force Gauge Model II. Weights of egg main parts (albumen, yolk and shell) were measured by Mettler Toledo PB1502-S scales. Shell thickness was measured by electronic micrometer of the 0.001 mm precision level, in the equatorial zone of egg shell. Percentage of albumen, yolk and shell portion was calculated mathematically out of data obtained by measuring weights of egg main parts in relation to the whole egg. VN was calculated out of refractive index of albumen and yolk (*Janke* and *Jirak*, 1934), applying the following formula: VN=1000 x (refractive index of yolk – refractive index of albumen). Protein and dry matter contents were determined on samples of each weight grade group according to the *Lowry* method (1951), in the UV/VIS JENWAY 6305 spectrophotometer. Data on protein content were further used in evaluation of different weight grade eggs. Research results were obtained by applying arithmetic mean ($\bar{x}$), standard deviation (s), standard error of arithmetic mean ($s\bar{x}$) and coefficient of variance (Cv%), and processed in ANOVA. Differences among different weight grade eggs were determined by the t-test at the significance level of 5% ($P<0.05$) in the software Statistica 7.1 (StatSoft, Inc. 1984–2005).

RESULTS AND DISCUSSION

The poultry industry pays great attention to production technology and high quality products. Contemporary consumers are well informed and know that quality of production is directly related to quality of commercial products. As large supermarkets are being supplied with eggs from numerous small farms, it is of use to be aware of the quality of eggs. *Table 2* presents quality traits of eggs of S, A and B weight grades. If considering the egg appearance, i.e. form index, shell firmness and thickness, no statistically significant differences ($P>0.05$) were determined among investigated egg groups. The highest form index value was obtained in the S weight grade eggs (78.27), followed by eggs of A (77.79) and B (77.21) weight grade. These results correspond with the results obtained by *Casiraghi et al*. (2005) and *Lukić et al*. (2004). Obtained results were as expected, as egg groups were formed on the basis of weight grades, so the highest form index was expected for eggs of the S grade. If comparing results on the egg shell thickness stated by *Casiraghi et al*. (2005) (Small=0.4 mm; Medium=0.42 mm; Large=0.42 mm and Extra Large=0.41 mm), obtained data in our research are lower (B=0.367 mm; A=0.371 mm; S=0.369 mm), however, they are in accordance with results published by *Lukić et al.* (2004) and *Škrbić et al.* (2004). Egg shell firmness was similar in all investigated eggs (S=3.59 kg/cm^2; A=3.54 kg/cm^2; B=3.40 kg/cm^2). Referring to the egg freshness trait, statistically significant difference ($P<0.05$) was determined only for egg yolk color of the S weight grade in comparison to the A and B weight grade eggs (S=13.08; A=12.83; B=12.76). In his research, *Hernandez* (2005) found out that consumers preferred eggs of intensive yolk color. This especially refers to Germans, as they consider a quality egg yolk to have the color intensity between 12 and 14. Following that fact, eggs analyzed in our research are satisfactory for the European market with respect to their yolk color. *However*, *Škrbić et al*. (2004) and *Lukić et al*. (2004) in their researches into the quality of commercial eggs obtained lower values for yolk color (9.21–10.25). Other indicators of egg freshness: albumen height, HU, pH of yolk and albumen, and VN did not exhibit statistically significant differences ($P>0.05$). Similar values referring to albumen height (S=7.88 mm; B=7.77 mm) and HU (S=86.7; B=87.84) were determined in eggs of S and B groups, while eggs of the A group had slightly lower values of that trait (7.35 mm; 84.36). The value referring to pH of yolk in all analyzed eggs was 6.07, while the slight difference was determined in pH of albumen (S=8.45, A=8.49, and B=8.42). Similar VN was obtained from eggs of S and A weight grade (62.47 and 62.62, respectively), while eggs of the B grade have slightly lower value (61.47). These results are in accordance with results obtained by *Kralik* (1976), who determined the average VN of 2-day old eggs to be 63.14. Obtained values of investigated traits indicate that the analyzed eggs were of extra quality. Portions of albumen, yolk and shell (%) in eggs of different weight grades are shown in the *Figure 1*. Higher portions of albumen (S=62.79%; A=62.81%; B=62.74) and yolk (S=25.4%; A=25.17%; B=25.17) were obtained in eggs of the S and A weight grades, while the B grade eggs had higher portion of shell (S=11.81%; A=11.66%; B=12.09%). Stated differences among groups were not statistically significant (P>0.05).

Table 2

Quality traits of eggs

Indicator	Statistical parameter	S	A	B	P-value
Egg weight, g	$\bar{x}$	66.81[a]	62.46[b]	58.06[c]	<0.001
	s	1.38	1.58	1.10	
	$s\bar{x}$	0.19	0.19	0.20	
	Cv%	2.07	2.53	1.89	
Form index	$\bar{x}$	78.27	77.79	77.21	0.315
	s	4.29	2.111	2.25	
	$s\bar{x}$	0.61	0.25	0.41	
	Cv%	5.48	2.71	2.92	
Yolk color	$\bar{x}$	13.08[a]	12.83[b]	12.76[b]	0.022
	s	0.49	0.65	0.43	
	$s\bar{x}$	0.07	0.08	0.08	
	Cv%	3.73	5.07	3.37	
Albumen height, mm	$\bar{x}$	7.88	7.35	7.77	0.082
	s	1.37	1.34	1.36	
	$s\bar{x}$	0.19	0.16	0.25	
	Cv%	17.33	18.23	17.51	
Shell firmness (kg/cm^2)	$\bar{x}$	3.59	3.54	3.40	0.442
	s	0.57	0.67	0.75	
	$s\bar{x}$	0.08	0.08	0.14	
	Cv%	15.77	18.85	22.03	
Shell thickness, mm	$\bar{x}$	0.369	0.371	0.367	0.677
	s	0.02	0.02	0.02	
	$s\bar{x}$	0.003	0.002	0.004	
	Cv%	5.15	5.56	5.81	
HU	$\bar{x}$	86.7	84.36	87.84	0.148
	s	9.43	9.08	8.07	
	$s\bar{x}$	1.33	1.07	1.47	
	Cv%	10.87	10.76	9.19	
Albumen, pH	$\bar{x}$	8.45	8.49	8.42	0.226
	s	0.19	0.22	0.15	
	$s\bar{x}$	0.03	0.03	0.03	
	Cv%	2.31	2.59	1.78	
Yolk, pH	$\bar{x}$	6.07	6.07	6.07	0.879
	s	0.07	0.08	0.06	
	$s\bar{x}$	0.01	0.01	0.01	
	Cv%	1.18	1.38	0.94	
Value number (VN)	$\bar{x}$	62.47	62.62	61.47	0.089
	s	2.71	2.51	1.71	
	$s\bar{x}$	0.38	0.30	0.31	
	Cv%	4.34	4.01	2.78	

a, b, c : P<0.05

Figure 1

Portions of main parts in eggs

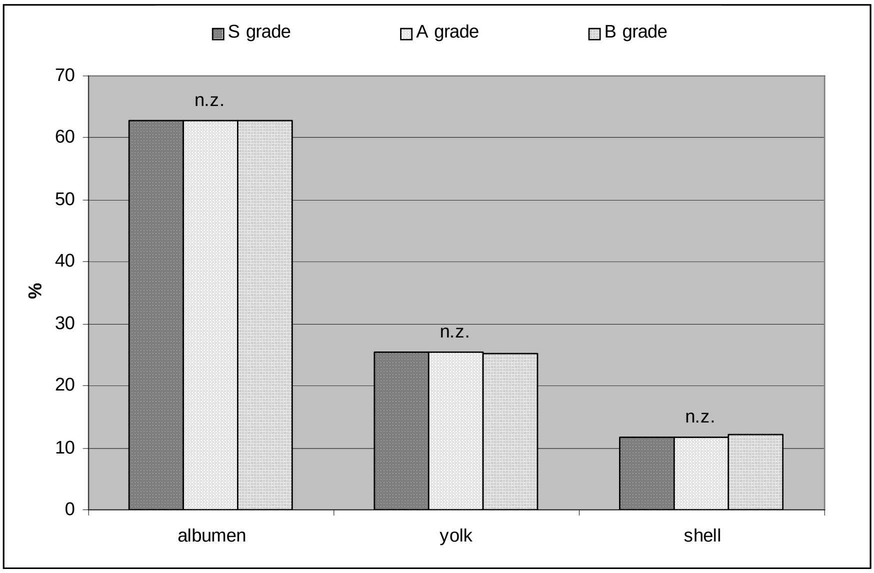

Table 3 presents an overview of prices per one egg, its edible part, protein and dry matter content, all depending on particular weight grade. It is obvious that the price is increased proportionally to the egg weight (S=0.135 €; A=0.122 €; B=0.108 €), however, edible part of egg, protein and dry matter are the cheapest in eggs of B weight grade. For example, for 100 g of protein in the S grade eggs, a consumer will pay 0.24 € more than for the same amount of protein in eggs of the B weight grade.

Table 3

Mean prices (€) of different weight grades

Weight grades of eggs	Price per egg	Price per 100 g of edible part	Price per 100 g of protein	Price per 100 g of dry matter
S	0.135	0.23	1.96	2.83
A	0.122	0.22	1.83	2.50
B	0.108	0.19	1.72	1.86

CONCLUSION

It is to conclude that B grade eggs have the most favorable pH value of albumen and the highest HU, and are the cheapest source of animal protein if respecting their price and protein content in yolk and albumen.

This research can be of use for consumers in their selection of different weight grade eggs, as it provides an insight into which eggs on our market are the cheapest and of the best quality.

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Effect of added copper and full fat soybean meal on growth performance and carcass properties in broiler chickens

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ABSTRACT

A 2×3 factorial experiment was conducted using a total of 504, 1 day old, Cob 500® commercial broilers to determine the effect of added copper (0 or 250 ppm) and full fat soybean meal level (10, 20 and 30%) on the growth performance and carcass properties in broiler chickens. The chickens fed 250 ppm copper as copper sulfate had lower (P<0.05) average daily gain and lower finally average body weight then chickens fed no added copper diets. Also, added copper impaired (P<0.05) feed conversion ratio during 49-d experimental period. The chickens fed with 30% full fat soybean meal in diet had higher (P<0.01) average daily gain during day 7 to 49 and higher (P<0.01) average body weight at day 49 then those consuming other diets. The addition of 250 ppm of copper improved dressing percentage (P<0.01), increased (P<0.05) lightness of breast muscle and tended to reduce abdominal fat content (P<0.06). The results indicate that the addition of 250 ppm copper to broiler diets have adverse effect on growth performance, but improve dressing percentage and decrease abdominal fat content. Using a 30% of full fat soybean meal in a broiler diet improves growth performance without negative effect on dressing percentage and abdominal fat content in 49 days fattening period.
(Keywords: copper, full fat soybean meal, growth performance, broiler chickens)

INTRODUCTION

Copper (Cu) is the third most abundant essential trace element in animals, after Iron and Zinc. As a part of numbers enzymes, copper is required for a number of physiological functions mostly related to catalytic agents in the active sites of cuproenzymes (*McDowell*, 1992). Numerous investigators have reported that addition of copper above dietary requirements, so called pharmacological level, can stimulate growth and feed efficiency in poultry and alters lipid and cholesterol metabolism (*Pesti* and *Bakalli*, 1996; *Konjfuca et al*., 1997). Copper is both an essential and a toxic element. Excessive feed copper intake results in morphological and functional changes in visceral organs and may even cause death. Several studies with poultry showed that high levels of dietary copper can cause gizzard lining erosion, proventriculitis (*Wideman et al*., 1996), lesions in the oral cavity, tongue and pharynx (*Chiou et al*., 1999) and changes in visceral organs weights (*Jackson et al*., 1979). It has been also reported that the consumption of high fat diets deficient in copper had detrimental effects on copper nutritional status, intermediary metabolism and production performance (*Wapnir* and

Devas, 1995). There is no information about the interaction between high dietary level of fat (vegetable oils) and copper supplementation on growth performance and processing characteristics in broiler chickens. In nursery pigs, *Dove* and *Haydon* (1992) observed an interaction between Cu levels and addition of animal fat. They reported that young pigs fed the diet containing 250 ppm of added copper and 5% added dietary animal fat had increased growth performance, whereas pigs fed the diet containing only 5% added animal fat had decreased growth performance. The objective of the current study was to determine the effect of growth stimulating level of copper (250 ppm) and different levels of full fat soybean meal on growth performance and carcass properties in broiler chickens.

MATERIALS AND METHODS

Birds and diets

A total of 504 Cobb 500®, one day old commercial chickens were purchased from a local hatchery and kept on floor covered with wood shaving in environmentally controlled house during winter. Temperature was set at 1 day old at 30 °C during the first week, and gradually lowered by 2 °C per 2 day until a temperature of 20 °C was reached at the age of 28. The lighting schedule provided 23 h light per day. Until the 7 d of age a standard prestarter diet was provided *ad libitum*. At 7 days of age, birds were individually weighed and randomly allotted by body weight to one of six dietary treatments. Each dietary treatment was randomly allocated to seven replicates of 12 birds each. Experimental treatments were arranged as a 2×3 factorial with two levels of copper (0 or 250 ppm as $CuSO_4 \cdot 5H_2O$) and three levels of full fat soybean meal (10, 20 or 30%). Corn, blend of sunflower and rape seed oil and soybean meal was replaced by full fat soybean meal to keep similar levels of energy and protein among diets. Nutrients levels met at least 90% of the minimum nutritional requirement for broiler chickens as set by the *National Research Council* (1994). The chicken fed mesh starter diets from 8 to 21 day of age followed by feeding a finisher diet from 22 to 49 day. Access to feed was discontinued approximately 16 h before slaughter at 49 d of age. Ingredients and chemical composition of the experimental starter and finisher diets are shown in *Table 1*. Experimental diets and tap water were available *ad libitum.*

Record keeping, sample collection and analysis

Individual body weight (BW) and feed intake (FI) were recorded and corrected for mortality on day 7, 21, 42 and 49 of age. At the end of the experiment, two broilers with average body weights were selected, individually weighed and sacrificed by cervical dislocation and then were immediately bled. After evisceration proventriculus, gizzard, pancreas and liver were removed and weighed. Carcass yield and abdominal fat content were also determined. Abdominal fat was defined as the fat surrounding the gizzard extending within the ischium and surrounding the *Bursa Fabricii*, cloaca and adjacent abdominal muscle. On the breast muscle, after 10 min blooming time, meat color was measured using Minolta CR-410 chrome meter.

Statistical analysis

The data were analyzed as a 2×3 factorial arrangement with the cage as an experimental unit (n=7) using the GLM procedure of SAS (*SAS*, 1999). The model included the main effects of full fat soybean meal (FFSBM) and dietary copper concentration, and their interaction. The effects were considered significant if $P<0.05$.

Table 1

Composition of experimental diets

	Broiler starter[a]			Broiler finisher[b]		
Full Fat Soya, %	10	20	30	10	20	30
Corn	48.00	46.80	44.30	59.00	57.60	55.60
Full fat soybean	10.00	20.00	30.00	10.00	20.00	30.00
Soybean meal (44%)	34.00	27.50	21.00	24.00	17.00	10.00
Vegetable oil	2.00	1.00	0.00	2.00	1.00	0.00
DL methionine	0.30	0.30	0.30	0.30	0.30	0.30
HCl Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Limestone	0.90	0.90	0.90	0.60	0.60	0.60
Monocalcium phosphate	1.40	1.40	1.40	1.40	1.40	1.40
Sodium chloride	0.60	0.60	0.60	0.60	0.60	0.60
Ca-formiat	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin-mineral premix[c]	0.50	0.50	0.50	0.50	0.50	0.50
$CuSO_4{\cdot}5H_2O$[d]	-	-	-	-	-	-
Calculated nutritive value[e]						
ME (MJ/kg)	12.19	12.26	12.34	12.70	12.78	12.88
Crude protein, %	22.99	23.12	23.34	19.27	19.26	19.26
Crude fat, %	6.05	6.94	7.84	6.15	7.11	7.90
Crude fiber, %	3.50	3.58	3.66	3.18	3.25	3.31
Lysine, %	1.37	1.39	1.40	1.11	1.11	1.12
Methionine + cysteine, %	0.96	0.97	0.98	0.86	0.87	0.87
Treonine, %	0.80	0.81	0.82	0.68	0.68	0.68
Tryptophane, %	0.28	0.28	0.29	0.22	0.22	0.23
Calcium, %	0.91	0.94	0.96	0.80	0.80	0.81
Phosphorus , %	0.70	0.73	0.74	0.68	0.69	0.69

[a]Fed from 7 to 21 day of age; [b]Fed from 22 to 49 day of age; [c]Vitamin-mineral premix provided per kg of diet: vitamin A, 15 000 UI; vitamin D_3, 2 000 UI; vitamin E, 30 mg; vitamin K_3, 2 mg; vitamin B_1, 1 mg; vitamin B_2, 6 mg; vitamin B_6, 3 mg; Vitamin B_{12}, 10 µg; biotin, 100 µg; niacin 30 mg; pantothenic acid, 12 mg; folic acid, 0,5 mg; Fe, 50 mg; Cu, 8 mg; Mn, 80 mg; Zn 50 mg; J, 0,5 mg; Co, 0,2 mg; Se, 0,15 mg; [d]$CuSO_4{\cdot}5H_2O$ contains 25,0% Cu, and was substituted for 0.10% corn in high-Cu diets to provide 250 ppm Cu; [e]Calculated on the basis of composition of Feedstuffs, *NRC* (1994).

RESULTS AND DISCUSSION

In the first 7 day dead or underfed chickens were not observed, therefore all 504 chickens were included in the experiment. Overall mortality was 0.8% for the 49 d experimental period, which was within the expected loss for Cob 500® broilers up to 49 day of age. In starter period (7–21 days) one broiler died in group with added copper and 10% of FFSBM in the diet. In period from 22 to 42 day of fattening no chickens died, in the late finishing period (43 to 49 days) three chickens died, one in group with no added copper and 10% FFSBM and one in both groups with added copper and 20 and 30% FFSBM in broilers diet. There is no effect of dietary treatment on survival rate, but higher mortality was observed with prolongation of fattening period from 42 to 49 days.

The effects of added copper and FFSBM level on growth performance are shown in *Table 2*.

Table 2

Effect of added copper and full fat soybean meal level on growth performance in broiler chickens[a]

Copper	0 ppm			250 ppm			SEM	Cu (C)	Soya (S)	CxS
FFS (%)	10	20	30	10	20	30				
Body weight (g)										
7 d	151.1	151.3	150.9	151.1	152.3	151.6	1.89	NS	NS	NS
21 d	647.8	662.6	668.9	648.1	654.3	639.3	9.02	0.10	NS	NS
42 d	2107.9	2130.5	2220.4	2080.6	2049.2	2058.8	31.87	0.01	NS	NS
49 d	2613.0	2615.4	2772.5	2527.9	2529.8	2568.4	37.97	0.01	0.01	NS
Weight gain (g/day)										
7–21 d	35.5	36.5	37.0	35.4	35.8	35.0	0.55	0.05	NS	NS
21–42 d	69.5	69.9	73.9	68.14	66.4	67.6	1.38	0.01	NS	NS
7–42 d	55.9	56.5	59.1	55.1	54.2	54.5	0.89	0.01	NS	NS
7–49 d	58.6	58.7	62.4	56.5	56.6	57.6	0.89	0.01	0.01	NS
Feed intake (g)										
7–21 d	67.0	65.4	67.7	67.5	67.8	65.5	1.52	NS	NS	NS
21–42 d	128.8	128.1	128.6	126.4	128.3	121.3	2.81	NS	NS	NS
7–42 d	104.1	103.0	104.2	102.9	104.1	97.8	2.02	NS	NS	NS
7–49 d	119.6	118.2	119.6	117.9	119.9	114.5	2.48	NS	NS	NS
Feed/gain (g/g)										
7–21 d	1.89	1.79	1.83	1.91	1.89	1.79	0.04	NS	NS	NS
21–42 d	1.85	1.83	1.74	1.85	1.93	1.80	0.04	0.08	0.01	NS
7–42 d	1.86	1.82	1.77	1.87	1.92	1.79	0.03	0.09	0.01	NS
7–49 d	2.04	2.02	1.92	2.08	2.12	1.99	0.04	0.05	0.01	NS

[a]Least square means representing 7 replications per treatment; SEM: Standard Error Mean; NS: Not Significant; FFS: full fat soya.

There was no copper x FFSBM interactions for any of the performance characteristics measured during the experimental period (P>0.05). Added copper had significant effect on daily gain (DG) during all phases of the experiment. Also, copper affected BW at day 42 and day 49 and tended to affect (P<0.09) BW at day 21. Chicks fed 250 ppm copper as copper sulfate had lower (P<0.05) DG and lower finally BW then chicks fed no added copper diets. During 7 to 21 day, the addition of copper had no effect on the efficiency of feed utilization. However, feed conversion ratio (FCR) was impaired (p<0.05) by the addition of copper during 49-d experimental period. On the contrary to these results, *Miles et al*. (1998) reported that BW and FCR did not differ in chicks fed up to 400 ppm copper from copper sulfate. Also, *Pesti* and *Bakalli* (1996) reported that 125 or 250 ppm added copper into the diet had growth promoting effect, but 375 ppm added copper provided no further benefit. High level of added copper as copper sulfate into chicken diet could have a growth depression effect (*Wang et al*., 1987). Similar to our results, *Banks et al*. (2004) founded lower DG and BW in chicks fed 250 ppm copper as copper sulfate from 9 to 22 day of age. The same authors explained this with reduction in feed intake and thus WG of chickens. In our study, the addition of copper had no effect on daily feed intake during the 49-d experiment. Previous results indicated that addition 450 ppm copper as copper sulfate into diets decrease feed intake of chickens vs. those fed 300 ppm copper or less.

The FFSBM affected DG and BW if fattening period is 49, but not 42 days. The chickens fed 30% FFSBM in diet had higher DG during day 7 to 49 and higher BW at day 49 then those consuming other diets. These results may indicate that with prolongation of fattening period from 42 to 49 days the genetic potential for maximum growth of broilers could be limited with energy level in diet. As expected, increasing the FFSBM level and thus higher dietary energy content improve FCR.

Table 3

Effect of added copper and full fat soybean meal level on processing characteristics, meat color and visceral organs weight in broiler chickens [a]

Copper (mg/kg)	**0**			**250**			**SEM**	**Cu (C)**	**Soya (S)**	**CxS**
FFS (%)	10	20	30	10	20	30				
Dressing, %	72.7	71.4	71.3	76.7	74.9	74.5	2.1	0.01	NS	NS
AF, %	1.92	1.58	1.74	1.19	1.52	1.36	0.24	0.06	NS	NS
Meat color										
L	56.60	55.71	55.06	58.58	57.70	55.58	0.73	0.01	0.01	NS
a	11.65	11.98	11.57	11.17	11.01	11.34	0.58	NS	NS	NS
b	12.15	11.86	13.24	10.90	13.60	11.86	1.02	NS	NS	NS
Visceral organs weight (%)										
Gizzard	1.43	1.50	1.35	1.66	1.67	1.65	0.09	0.01	NS	NS
PV	0.29	0.29	0,19	0.31	0.32	0.21	0.02	NS	0.01	NS
Heart	0.48	0.45	0.37	0.51	0.46	0.35	0.03	NS	0.01	NS
Liver	2.34	2.33	2.14	2.50	2.49	2.41	0.10	0.05	NS	NS
Pancreas	0.23	0.22	0.19	0.22	0.23	0.21	0.01	NS	0.07	NS

[a] Least Square Means; SEM: Standard Error Mean; NS: Not Significant; FFS: full fat soya; AF: Abdominal fat; PV: Proventriculus.

The copper addition improved dressing percentage (P<0.01) and tended to reduce abdominal fat content (P<0.06). Similar findings have been observed for the comparison of abdominal fat content in chickens fed diets containing 125 and 250 mg Cu/kg of added copper (*Tangtaweewipat*, 2004). It has been reported that pharmacological concentrations of copper may altered lipid and cholesterol metabolism in rats and chickens. *Konjfuca et al.* (1997) observed reduction in fatty acid synthetase activity in chicks fed supplemented copper. The decrease in fatty acid synthesis could explain the reduction in abdominal fat deposition. Also, the observed reduction in abdominal fat content in copper supplemented chickens could be caused by reduced growth performance observed in these chickens.

The copper addition had significant effect (P<0.05) on gizzard and liver weight (*Table 3*). The increase gizzard and liver weight with addition of 250 mg Cu/kg diet were in agreement with a study in laying hens fed 400 mg supplemented Cu/kg diet or higher (*Jackson et al*., 1979). On the contrary to our results, *Tangtaweewipat* (2004) did not find any significant effect of added 125 or 250 mg Cu/kg diet on visceral organs weights in broiler chickens. FFSBM affected proventriculus and heart weight. The chickens fed 30% FFSBM in diet had lower heart and proventriculus weight then those consuming other diets.

Meat color is an important criterion that can be used by consumers to evaluate meat quality. Lightness (L* value) is important in white muscles and correlate with initial pH and drip loss (*Barbut*, 1997). In our study, L* value was affected by added copper and FFSBM. The copper addition increased ($P<0.05$) lightness of breast muscle, whereas higher level of FFSBM decreased it ($P<0.01$).

CONCLUSIONS

The results of the present study indicate that the addition of 250 mg Cu/kg diet in the form of copper sulfate has adverse effect on growth performance, but improves dressing percentage and tends to decrease abdominal fat content. Using the 30% of FFSBM in a broiler diet improves growth performance without negative effect on dressing percentage and abdominal fat content in 49 days fattening period.

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Productive and reproductive traits of three different Italian poultry species involved in an *in-situ* conservation programme

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ABSTRACT

A study was conducted in three different poultry species: duck (the Germanata Veneta and Mignon breeds), turkey (the Comune Bronzato and Ermellinato di Rovigo breeds) and guinea fowl (the Camosciata breed) to assess productive and reproductive traits of these species that are involved in a conservation and valorisation programme applied to the indigenous Veneto poultry breed. The productive characterisation was carried out during 2005 involving 510 animals while the reproductive traits were studied from 2003 to 2005 on 335 batches. Evaluation of performance traits was based on average daily gain and commercial live weight while reproductive traits were evaluated on percentages of fertility and hatchability. Analysis of variance were performed for production and reproduction traits. Breed, sex and conservation nucleus were significant sources of variation for productive traits while for fertility and hatchability percentages breed and year were the variation sources. Fertility percentages ranged between 38 to 68 respectively for Camosciata and Comune Bronzato breeds. Between 2003 to 2005 the fertility percentages improved for all local Veneto species. The Mignon and Camosciata breed did not show differences between males and females for average daily gain while the Germanata Veneta breed, Comune Bronzato and Ermellinato di Rovigo turkey breeds showed an evident sexual dimorphism.
(Keywords: Poultry, local breed, productive and reproductive traits; conservation programme)

INTRODUCTION

Animal genetic resources are the base for livestock production and development. Genetic diversity enables farmers and breeders to use a wide range of production environments and to develop diverse products to meet the needs of local communities. Moreover, it allows farmers and breeders to respond to the changing environmental conditions and consumer demands. Consequently, the contribution of animal genetic diversity in agriculture, economic development and management resources plays a fundamental role for their conservation. At the same time, being an integral component in many social and cultural traditions, diversity contributes both to the individual and community identity. For all these reasons in many countries the interest and the conservation programmes for safeguarding animal genetic resources are sensitively increasing (FAO 2004). The Co.Va. project (Conservazione e Valorizzazione di Razze Avicole Locali Venete) is an example of a conservation scheme that started in 2000 with funds of Veneto Region of Italy. This programme is the first one that has been carried out in Italy as an *in situ* marker assisted conservation scheme using genetic markers for the conservation of animal biodiversity (*Cassandro et al*., 2004). AFLP markers were used to perform genetic characterisation (*Targhetta et al*., 2005) and to monitor expected heterozigosity (*De Marchi et al*., 2006). This project has involved three organic farms located in different areas of the region.

The studied local duck breeds are the Germanata Veneta (GV) and Mignon (M); the local turkey breeds are the Comune Bronzato (CB) and Ermellinato di Rovigo (ER); the local guinea fowl breed is the Camosciata (C) and they are dual-purpose breeds for meat and egg production (*De Marchi et al*., 2005). The GV duck was derived from the Real German, its coloration and form are unchanged. This breed is very rustic, and the female can be crossed with the Barberia duck to produce fat liver for pate. The M is a small white duck with yellow legs, beak and skin and it is found in the southern and eastern part of the Veneto region. The CB turkey is a small breed. The breast, neck, shoulders, and rump are black with rainbow reflexes. The ER turkey was derived from a mutation in offspring of crosses of local birds to the American Narraganset breed in 1958 and was then selected for increase performance (*De Marchi et al*., 2005). The C guinea fowl was developed in 1922 (*Veneto Agricoltura*, 2004). The neck and throat skin are blackish, the feathers are white with pearl stains, and the tarsus coloration varies from orange to grey. The demand for products from the Veneto poultry breeds has also increased because of their perceived image as a source of nutritious and healthy natural products from birds that are reared in a clean and natural environment with no industrial residues. In developing systems of breeding, production and marketing for the Veneto avian breeds, emphasis was placed on an organic system of production including housing in an indoor pen with access to a grass paddock.

Aim of this study was the characterisation of productive and reproductive traits of three different poultry species involved in a conservation and valorisation plan applied to the indigenous Veneto poultry breeds.

MATERIAL AND METHOD

The productive characterisation was carried out in 2005. Five hundred and ten animals were branded with wing tags at hatch and reared in an indoor pen with an open grass paddock in the same period (from March to November) in three different conservation nucleus located in mountain (Feltre), hill (Montebelluna) and plain (Ceregnano) areas of the Veneto region of Italy.

In the Feltre conservation nucleus were reared the GV, C, CB and ER breeds, in the Montebelluna the GV, M, C, CB and ER, while in the Ceregnano the C, CB and ER breeds.

In each nucleus birds were fed *ad libitum* on complete feeds of similar chemical composition. All animals were hatched following a reproduction scheme developed in the same time in all conservation nucleus and weighted in the selection period from October to November. The average daily gain (g/d) was calculated as ratio between commercial live weight (g) and age (d).

A total of 24,876 eggs was incubated in 335 batches (from March to May) during 2003, 2004 and 2005 to determine the fertility and hatchability. Candling of then incubate eggs was done at 14 days after incubation. The percentage fertility of the eggs was calculated as follows: (Te–Ie/Te)×100 where Te was the total number of eggs incubated and Ie the total number of infertile eggs; while the percentage hatchability of the eggs was calculated as follows: (He/Ve)×100, where He was the total number of hatched eggs and Ve the total number of viable eggs (after the first candling) according to *Msoffe et al.* (2004).

Using a GLM procedure (*SAS*, 1999), analysis of variance of commercial live weight and average daily gain was conducted considering the following sources of variation: breed and nucleus combined (BN), sex (S), and the effect of their interactions (BN×S). The least square means solutions of the combined effect were used to estimate sex effect using the contrast statement. Also for the reproductive traits an analysis of variance was performed using the following sources of variation: year and breed combined (YB), nucleus (N), and the effect of their interactions (YB×N). The least square means solutions of the combined effect were used to estimate year effect using the contrast statement.

RESULTS AND DISCUSSION

The GV breed showed an higher average daily gain than M breed (*Table 1*). The commercial live weight (g) of GV breed was 2251±166 and 2545±136 respectively for females and males while the M breed showed a similar commercial live weight (g) ranging between 877±109 to 971±102 respectively for females and males. The GV average daily gain (g/d) showed an important difference between males and females (16.03 vs 19.42, respectively). The CB turkey breed showed a lower commercial live weight respect to the ER breed according to the value reported by *De Marchi et al.* (2005). The turkey breeds showed a consistent difference of average daily gain (g/d) between females and males (19.62 vs 28.82) and (24.22 vs 33.95), respectively for CB and ER breeds (*Table 1*). The C breed showed a low commercial live weight (g) 1406±142 and 1545±128 respectively for females and males, whit modest sexual dimorphism (*Table 1*). The values reported for the C, GV and M breed were similar to those reported by *De Marchi et al.* (2005) and *Veneto Agricoltura* (2004).

Table 1

Average daily gain (g/d) of the indigenous Veneto poultry breed

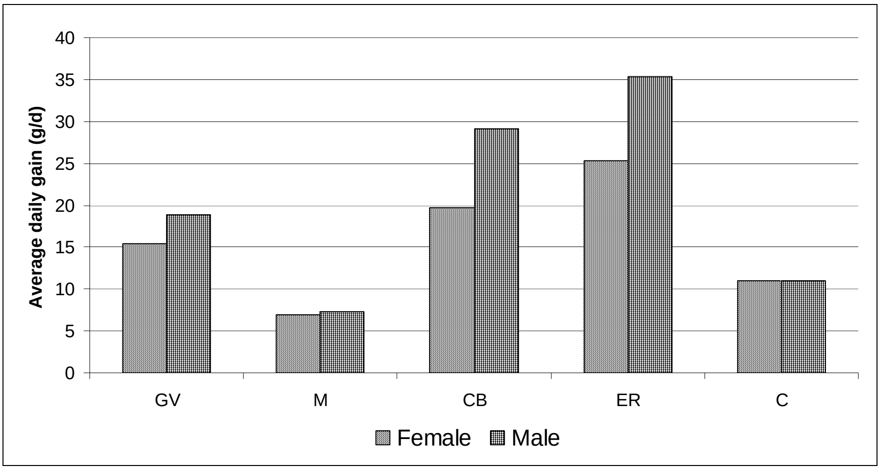
Breed	Average daily gain (g/d)			
	Female		Male	
	n	Means±SD	n	Means±SD
GV	40	16.03±1.60	27	19.42±2.44
M	30	6.87±1.47	20	7.30±1.04
CB	71	19.62±2.35	48	28.82±4.12
ER	74	24.22±4.07	40	33.95±6.23
C	94	11.06±1.60	66	10.99±1.95

GV: Germanata Veneta, M: Mignon, CB: Comune Bronzato, ER: Ermellinato di Rovigo, C: Camosciata.

Statistical models used for the study of productive traits were adequate, evidencing a high determination coefficient (R^2>0.90) and all effects were highly significant ($P<0.001$). In the *Figure 1* are shown the least square means of average daily gain. The M and C breed did not show any statistical difference between sex, while that difference was important in the CB and ER turkey breeds. Males of the GV duck breed showed a better average daily gain (g/d) respect to females (18.8 vs 15.4). Average daily gains (g/d) for the three species were higher in the Castelfranco and Ceregnano nucleus than in Feltre (19.1 and 18.1 vs 14.5 respectively). Regarding C breed, average daily gain was 12.8, 10.6 and 9.4 g/d in Castelfranco, Ceregnano and Feltre nucleus respectively. For the CB and ER turkey breeds daily gains were similar in the Castelfranco (25.0 and 33.4 respectively) and Ceregnano nucleus (26.9 and 33.0 respectively), while was lower in the Feltre one (21.3 and 24.4 respectively).

Figure 1

Least square means of average daily gain (g/d) for the indigenous Veneto poultry breed



Statistical models used for the study of reproductive traits showed a determination coefficient range between 0.33 and 0.59 for hatchability and fertility values respectively. All effects were highly significant (P<0.001) for fertility percentages. Regarding hatchability percentage YB, N and YB x N were significant effects (P<0.001, P=0.002 and P=0.035 respectively).

Table 2

Descriptive statistics of reproductive traits for the indigenous Veneto poultry breed

Breed	Batches	Incubated eggs	Fertility	Hatchability
			Means±SD	Means±SD
GV	70	7689	0.57±0.16	0.63±0.15
M	72	7470	0.53±0.18	0.64±0.17
CB	80	4401	0.68±0.21	0.82±0.13
ER	72	1509	0.38±0.30	0.58±0.31
C	41	3807	0.67±0.25	0.59±0.17

In *Table 2* are shown the descriptive statistics of reproductive traits for the Veneto poultry breeds. The number of batches during 2003, 2004 and 2005 ranged between 41 to 80 respectively for the C and CB breed, while the number of incubated eggs ranged between 1509 to 7689 for the ER and GV respectively. The values of fertility ranged between 0.38 to 0.68 for the ER and CB, while the hatchability values ranged between 0.58 to 0.82 for the ER and CB respectively. The fertility and hatchability percentages of the C breed were similar to those reported for the local domestic fowl ecotype of Tanzania (*Msoffe et al.*, 2004). The low fertility found in these species was probably caused by confinement stress that prevented the males from expressing their optimal reproductive performances. There is also a possibility, that there were some deficiencies in the commercial feeds given to the animals since no attempt was made to evaluate the nutritional status of the feeds. Another reason might be the fact that eggs had to be stored for up to one week prior to the incubation. Although all the necessary storage precautions were taken, there was still a chance that fertility was lost during storage especially due to diurnal temperature variations. The hatchability percentage obtained in the current experiment was rather low compared to some previous studies. For instance *Wilson* (1979) observed mean hatchability values of 90% in the Sudanese local domestic fowls; *Barua* and *Yoshimura* (1997) reported values of 75% on the local Bangladesh domestic fowls. Similarly *Mwalusanya* (1998) reported hatchability in the free-range local domestic fowls of Tanzania to be over 80%. The fertility percentage of the GV and M breed were lower than those reported for the Muscovy duck breed (Nickolova 2005). The percentage of hatchability of the GV and M breed were lower respect to those reported for Turkish Pekin (78.5%), and the two local populations Boz (70.2%) and Yesilbas (73.0%) (*Isguzar*, 2005).

In the *Figure 2* and *Figure 3* are shown the variation of fertility and hatchability percentages between 2003 and 2005. The fertility percentage of the local species involved in this conservation programme did not show any variations from 2003 to 2005 with the exception of the ER breed that demonstrated a significant increase (P<0.001). The hatchability percentage of the GV and M decreased from 2003 to 2004 (62% vs 42%, P<0.027) and (59% vs 37%, P<0.016). The CB breed did not show any statistical variation of hatchability percentage from 2003 to 2005, while for ER breed there was a statistical difference from 2003 to 2004 (P<0.001).

Figure 2

Least square means of fertility percentage from 2003 to 2005 for the indigenous Veneto poultry breed

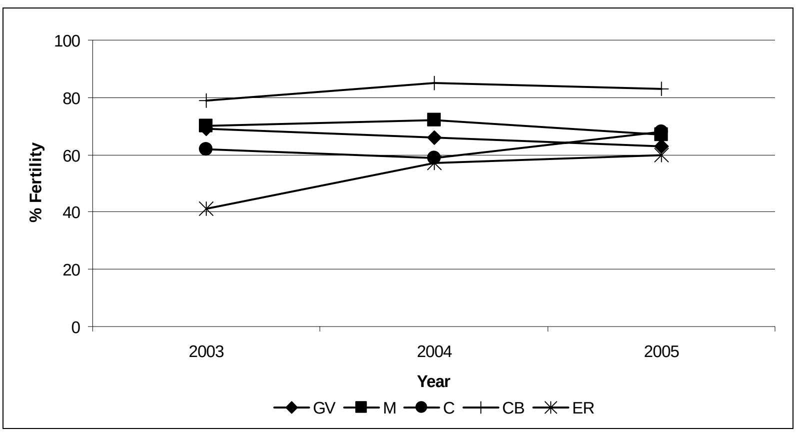
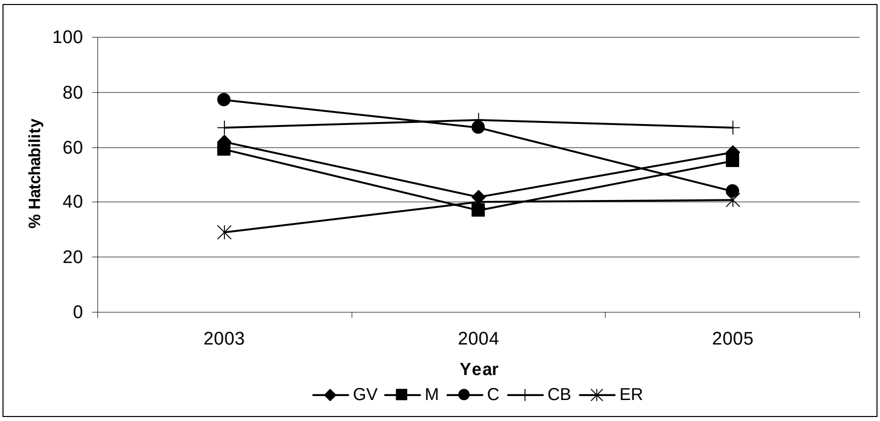


Figure 3

Least square means of hatchability percentage from 2003 to 2005 for the indigenous Veneto poultry breed



CONCLUSIONS

The results of this study confirmed the performance characteristics of the duck, guinea fowl and turkey Veneto poultry species reported in other studies. The breed, sex and conservation nucleus were significant sources of variation for commercial live weight and average daily gain. Regarding reproduction traits all breeds showed medium-low fertility and hatchability percentages. More studies should be conducted to deepen the reasons of the reduced reproductive performances.

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Animal welfare aspects of goose liver production without force feeding: selection possibilities for behaviour forms

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ABSTRACT

The objective of our experiment − accomplished between 2003–2004 − was to analyse the basic behaviour forms of the domestic geese (feed intake, playing, social behaviour, preening) in order to determine the selection possibilities for these behaviour forms. Because of the small dataset genetic parameters were estimated using Bayesian statistics through animal model. The heritability of certain behaviour forms was low which suggests that the analysed behaviour forms are mainly determined by environmental factors consequently small selection response can be expected. However, the time spent with feed intake (which is the most important trait from our viewpoint) showed heritability of 0.27–0.28 and might be a suitable selection criterion.
(Keywords: goose, behaviour, heritability, feeding, animal welfare)

INTRODUCTION

During the last years foie gras production has been increased worldwide but the majority of the production is confined to a few countries. From the 17000−18000 tonnes fatty liver (geese, ducks and mulard) annually produced worldwide (in the recent years) France is the largest producer sharing 80% of the total production. Concerning foie gras production in gees, Hungary is the largest producer giving 60% of the world's production (that is 9% of the world's total foie gras production; *Guy* és *Guémené*, 2004). The Hungarian production is export oriented (75−80%), the main target countries are France (65%) and Japan. In Hungary the conventional technology of fatty goose liver production is force-feeding (*Bogenfürst*, 1992). Because of the effective lobby of the animal welfare organisations, foie gras production based on force-feeding has to be replaced by alternative methods by 2015. The SCAHAW (1998) published a report in December 1998 on the welfare aspects of the production of foie gras in ducks and geese. In this report force-feeding was rejected as the suitable method of foie gras production. The final conclusion of the report was that force-feeding in its present form − as it damages the liver − was an unsuitable technology from the animal welfare aspects. Implementation of force-feeding was also unsuitable from animal welfare aspects due to the inconveniences caused by the pipe inserted into the oesophagus. Although experimental results of Hungarian researchers disproved the assumption that force-feeding damages the liver (*Locsmándi et al*., 2004) intensive research is being conducted aiming the replacement of this technology in order to produce foie gras (*Bogenfürst et al*., 2000). One alternative is the phased feeding that showed very promising results. However, application of this technology requires a goose genotype that not only responds favourable to phased feeding from the aspects of liver development but also capable to alter its feeding pattern and willing to consume large amount of feed in a relatively short time.

The objective of the present study was to examine the possible differences of the behaviour forms among the various goose genotypes and to estimate their heritability (especially for feed intake).

MATERIALS AND METHODS

Methodology of the present study was developed acoording to *Reiter* and *Bessei* (1995) and *Molnár et al*. (1998, 1999). The experimental work could be divided to three parts. The identity of parents of the studied individuals was crucial thus in the first phase of the experiment introduction of the breeding stock, individual egg collection and pedigree hatching were accomplished. Subsequently in the second phase of the experiment the hatched goslings were placed, their behaviour forms were determined, monitored and evaluated. Finally in the third phase of the experiment heritabilities of the various behaviour forms were estimated.

The breeding goose were placed to pens having a basic area of 1.3×2.5 m and the adjacent pens were divided by wire net. The applied stocking density was 0.8 m^2 which decreased to 0.54 m^2 after placing the nest to the pens. The applied technology corresponded to the intensive technology generally applied (*Bogenfürst*, 1992). The animals were feed with a commercial pellet. Hay was used for litter material, pellet and water were available from feeding-trough and open surface drinkers, respectively.

Hatching of the produced eggs was accomplished in three turns. The goslings with known parents could be placed to 16 pens. Five goslings were placed into a pen. Until the age of four weeks the goslings were placed to pens having a basic area of 1.5×1.5 m and OSB (Oriented Strand Board) lateral panels then they were moved to pens described above (3×2.5 m). Thus it was possible to maintain the stocking density defined by the keeping technology.

Until the thermoregulation system of the goslings was developed (the first two weeks) the appropriate temperature was provided using 250 W infra lamps. The pellet was available ad libitum from feeding-troughs. Once a week behaviour of the geese was recorded by digital cameras directly to personal computer. The behaviour forms were then counted in every minute. At a given time the occurrence and the frequency of the six behaviour forms were studied.

The investigated behaviour forms were: nutrition, drinking, resting, social behaviour, preening and playing. The definition of these behaviour forms were given by *Czakó* (1985).

Feeding consists of exploration, recognition, approaching and consumption. Exploration can be considered as the appetitive phase of nutrition triggered not directly by the stimulus of the feed (rather than the hunger). This is followed by the selection and consumption of the feed. The smell, taste and appearance play an important role. The intensive movements of the head (forward and back) makes clear distinction of nutrition from playing (with feed) possible.

Drinking is one of the behaviour forms connected with metabolism, its accomplishment characterise the different species, its rhythm depends from the amount of the available water and from internal causes like dehydration of the mucous membrane of the oral cavity or the throat. Drinking is performed through series of movements characterised by geese: dips the beak into the water then stretches forward the neck lifts up the head and using gravitation flows the water down through its throat. It helps to make a distinction between drinking and playing or bathing.

Resting is the rest phase of the motorium that can be identified from a special resting position. The resting period may be interrupted by preening and playing but the intensity of these behaviour forms are much lower than those times they performed separately. Adult geese rarely sit for resting in most cases they stand motionless.

Social behaviour can be considered as all the behaviour features that manifest among the individuals of the same population. In this study fighting, greeting and preening other individuals were classified into this category.

Preening is the cleaning and ordering of the integument that manifests not during resting but separately and showing high intensity. The behaviour is only classified into this category when it manifests towards the own plumage, preening other individuals are sorted to social behaviour form. Preening often coincide with bathing.

Playing is the collective term for various behaviour forms that play an important role in ontogenesis, development of regulating system and capability but do not have any practical purpose. In an environment of low stimuli they necessarily manifest with higher frequency. Playing can be realized with feed, water and with the equipments connected to feed and water replacement and consequently may cause wet litter. The playing caused by boredom can mainly explained by the fact that the geese kept under intensive technology cannot satisfy their grazing instinct.

Based ont he data recordings heritabilities of the various behaviour forms were estimated using Bayesian statistics (*Wickmann*, 1990) through animal model. Apart from the relationship among the individuals, genotype was also considered in the model. Heritability was determined by the posteriori distribution using Markov chain. The total number of chain elements was 60000 from which 10000 was used as burn in and only every 10th element was taken thus the final sample consisted of 5000 elements. The statistical analysis was carried out applying the GIBBS3F90 software, and the heritability distributions were depicted with the SPSS for Windows 10 (*SPSS Inc*., 1999).

RESULTS AND DISCUSSION

Heritability of the behaviour forms is given in *Table 1*.

Table 1

Heritability estimates of the behaviour forms

Trait	Mean	Median	$BHD_{0.95}$	k_1	k_2
feed intake	**0.2862**	**0.2737**	0.0077 0.5440	0.0625	0.5422
drinking	**0.2652**	**0.2510**	0.0074 0.5486	0.0485	0.5481
resting	0.0691	0.0532	0.0016 0.1874	0.0056	0.1870
playing	0.0700	0.0540	0.0007 0.1846	0.0055	0.1846
social behaviour	0.1181	0.0977	0.0030 0.2911	0.0110	0.2911
preening	0.1126	0.9449	0.0050 0.2718	0.0130	0.2714

$BHD_{0.95}$=high posterior density interval at a 95% of probability; k_1=limit of the interval containing a probability of 95%, [$k_{1,}$ 1]; k_2=limit of the interval containing a probability of 95%, [0, k_2].

Heritability estimates of the feed intake and drinking was moderate. There are traits like egg production used as selection criterion contrary to its low heritability (h^2=0.15–0.20) (*Dohy*, 1999). The relatively low heritabilities found by this study may be explained that they were estimated by animal model and it was generally observed that the traits show lower heritabilities based on animal model compared to estimates based on other procedures like correlation among full-sib groups. Heritability estimates of resting, playing, social behaviour and preening were low or negligible. Selection for these behaviour forms cannot be suggested as the expected selection response would be probably low and the traits have low functional value.

According to the knowledge of the authors selection based on behaviour forms of geese has not yet been published. On the contrary the behaviour of pigs was extensively analysed by several authors (*von Felde et al*., 1996; *Kalm et al*., 1996; *de Haer* and *de Vries*, 1993). In these studies the number of the recorded animals ranged between 1832–3188 and their behaviour was monitored for 10 weeks. Using computer chips for identification and special feeders made the monitoring of several behaviour forms (daily feed intake, feed intake per visit etc.) possible using group-penning. The total time spent on daily feed intake showed heritabilities of 0.24–0.45.

It has to be noted that from the several behaviour trait von *Felde et al*. (1996) suggested the total time spent on daily feed intake as a selection criterion trait as it was the only behaviour trait showed reasonable genetic correlation with daily feed intake (r_g=0.44) and average daily gain (r_g=0.32).

CONCLUSIONS

The main advantage of the application bayesian statistics for heritability estimation is that it is a highly suitable procedure for small datasets. The conclusions can be made on exact probabilities that give a good flexibility evaluating the results and the non-normal distribution of the traits can also be taken into account.

Among the studied behaviour forms the time spent for daily feed intake and drinking showed moderate heritabilities, from which the former traits may be suitable for selection.

Based on the results further experiments are planned to accomplish where a two-way selection will be carried out for the time spent for daily feed intake and the behaviour of the animals selected upwards and downwards will be compared.

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The bird flu in mind of Hungarian consumers- lesson and experiences of a direct-question survey

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ABSTRACT

The emergence of avian flu in Europe means new challenges for the Hungarian food chain, highlighting the importance of risk-communication to the consumers. Based on a direct-question survey in end of 2005, the article analyses the consumers' preference structure and risk-perception. Basic information source is the electronic and printed media, but – especially in case of lower qualified consumers – the effect of friends and relatives is considerable, too. The level of thrust is especially high in researchers and medical authorities. This fact should be more utilised in communication. The pro-active strategy of Hungarian Poultry Product Council can be evaluated as a positive one. Beside the negative effects (decreasing consumption), the avian flu problem enhances the importance of place of origin of products. The system of factors, influencing consumer behaviour was analysed by the Fishbein-Ajzen model. Utilising the structural equation approach, it was possible to determine the system factors, influencing the consumer behaviour. It became obvious, that the attitudes of consumer exercise a determining effect on the practical behaviour in poultry consumption.
(Keywords: consumer research, attitudes, Fishbein-Ajzen model)

INTRODUCTION

Most influenza viruses occur in birds, particularly the aquatic waterfowl that are their natural reservoir. Only a few types of influenza virus have circulated widely in humans. "Bird flu" refers colloquially to both influenza in birds and to instances when these avian viruses jump the species barrier to cause human disease. In 1997, a cluster of avian influenza due to influenza A (H5N1) occurred in people in Hong Kong (5). This outbreak was unique and alarming because it was the first recognized direct transmission of influenza from birds poultry to people, it involved a unique strain (H5N1),and it was highly fatal: Six of 18 (33%) recognized case patients died.

In second half of 2005 the avian flu virus has been detected Europe. Even the average consumers had got the knowledge of the emergence of this zoonose-risk. The level of public interest can be seen well on base of an extract from the Newsweek magazine article: „The world is on edge, stalked by a virus that travels the great migratory flyways and kills where it lands. After incubating in 1997 in East Asia, where it was responsible for the death of 140 million birds (including those intentionally destroyed to stop its spread) and 68 people, the H5N1 variant of avian flu suddenly and mysteriously expanded its range this year, north to Mongolia and Siberia, then west into Ukraine, Croatia and Turkey. Through innumerable generations and hundreds of mutations, it maintained its extraordinary lethality, without yet evolving the ability to be transmitted directly between people. Almost all cases have involved people who came into close contact with chicken blood or droppings; when and if that changes, it could be the trigger for a global pandemic that, in a worst-case extrapolation from the toll of the 1918 Spanish flu, could kill 150 million people – 2.5 percent of the world's population – in a matter of months…. Governments–indeed, civilizations–have collapsed from less…”.

Aim of this article is to determine the Hungarian consumer information sources, attitudes and behavior after the emergence of avian virus in Europe. We have been tested four work hypotheses:

1. H1 The most important sources of information of Hungarian citizens are the TV channels and the electronic media;
2. H2 The avian flu increased the importance of country of origin in case of majority of consumers;
3. H3 There is a rather low level of confidence among Hungarian consumers towards the different professional and governmental authorities;
4. H4 The food consumer behaviour can be explained by the general theory of the well-known Fishbein-Ajzen model of planned behaviour.

In trying to understand the basis of consumer behaviours psychologists, marketing specialists and health educators have together gathered an impressive list of factors and constructs which at one time or another have been said to be relevant, but these factors are hard to operationalise. That’s why we have a rather simple, but easily operationalisable method of investigation: the Fisbein-Ajzen (*Collins* and *Wugelther*, 1992; *Fishbein* and *Ajzen*, 1974) model. Searching the causes of human behaviour Ajzen and Fishbein state, that: "the ultimate determinants of any behaviour are the behavioural beliefs concerning its consequences and normative beliefs concerning the prescriptions of others" moreover "variables other than these two components (are) shown to affect behavioural intentions and overt behaviours indirectly by influencing one or both of the components". This certainly prunes radically the number of relevant factors, influencing consumer behaviour, that’s why this approach has been used to analyse the consumer behaviour. Behaviour is defined as "Observable acts ... that are studied in their own right". The model provides a framework to study attitudes toward behaviours. According to the theory, the most important determinant of a person's behaviour is behaviour intent. The individual's intention to perform a behaviour is a combination of attitude toward performing the behaviour and subjective norm. If a person perceives that the outcome from performing a behaviour is positive, she/he will have a positive attitude forward performing that behaviour. If relevant others see performing the behaviour as positive and the individual is motivated to meet the exceptions of relevant others, then a positive subjective norm is expected. Attitudes and subjective norm are measured on scales (as an example the Likert Scale) using phrases or terms such as like/unlike, good/bad, and agree/disagree. A positive product indicates behavioural intent (*Glanz et al*., 1997). The third determinant of behavioural intention is the perceived behavioural control. This perception can reflect past experiences, anticipation of upcoming circumstances, and the attitudes of the influential norms that surround the individual (*Mckenzie* and *Jurs*, 1993).

METHODS OF INVESTIGATIONS

Between October and November 2005 (after the publication of pieces of information on indication of avian flu in Turkey, Romania and Bulgaria) a series of focus-group interview has been carried out to determine the most important points of public interest, joining to avian flu problem. Based on these interviews, a questionnaire, containing 97 items has been compiled. The questionnaire consisted of closed questionnaires with purpose of facilitating of the anonym filling out. The respondents have been selected from participants of different meetings of a local rural library, form parents of students of a Budapest high school, as well as from relatives of students of different post-graduate courses of Corvinus University. Total number of respondents has been 526. The graphical representation of results has been realised by explorative data analysis (*Hajduné*, 2005). The boxes in the graphs represent the interquartile range, the thicker lines the mode of responses.

The basic socio-economic characteristic features of respondents are summarised in *Table 1*. The sample can't be considered as a representative one, but it seems to be suitable to determine the attitudes of Hungarian above average.

Table 1

The basic socio-demographical indicators of respondents

Gender	
Female	65%
Male	35%
Age	
Below 35	37%
36–50	41%
Above 50	12%

Place of living	
Budapest (capital of Hungary)	35%
Larger town (County centre)	20%
Small town	27%
Village	18%
Level of qualification	
Elementary school	18%
High school	44%
College, university	38%

RESULTS

The most important sources of information on avian flu for the respondents were the electronic and printed media (*Figure 1*). It is important to emphasise, that the relative importance of state and commercial television channels were practically the same. This highlights the responsibility of business oriented means of communication in information of consumers. Surprisingly, the importance of friends and relatives as sources of information was relatively high.

The level of confidence in different sources of information has shown considerable differences (*Figure 2*). The highest level of trust was in information from Hungarian scientists and competent medical authorities. The level of trust was considerable lower in case of Ministries. The emergence of avian flu has highlighted the importance of question of place of origin of the products. It can be estimated as a rather positive fact, that the products, bred or processed in Hungary have been preferred in an above average level (*Figure 3*).

The structural model describes two types of relationships: the relationships between observed variables and latent variables, and that among latent variables. The directly observed variables are indicated by ellipses. The continuous latent variables (attitude, norms, perceived control) are indicated by rectangles. The behaviour itself (marked by rectangle) has been measured by four indicators. The graph shows the unstandardized coefficients. Each unstandardized estimate represents the amount of change in the outcome variable as a function of a single unit change in the variable causing it. By definition, the first estimate in each group of variables is set as 1.

Figure 1

The relative importance of different sources of information, evaluated on a 1-5 scale

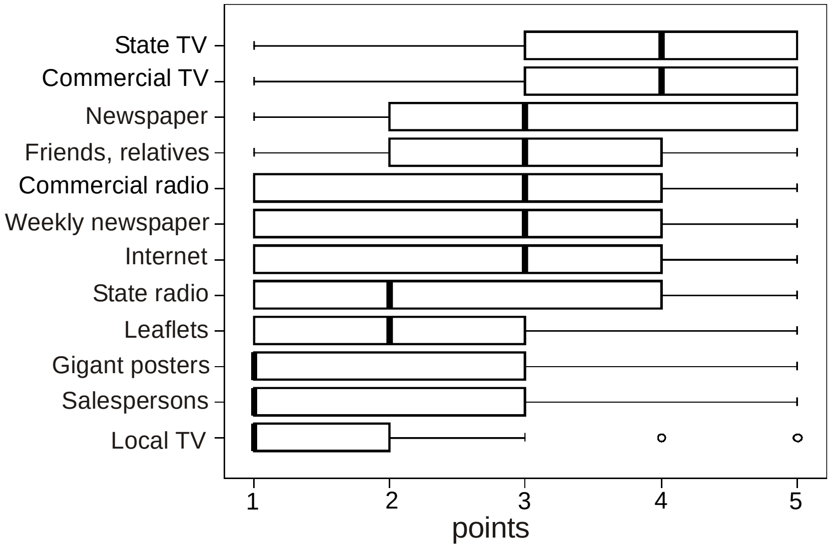


Figure 2

The level of trust in different sources of information on avian flu

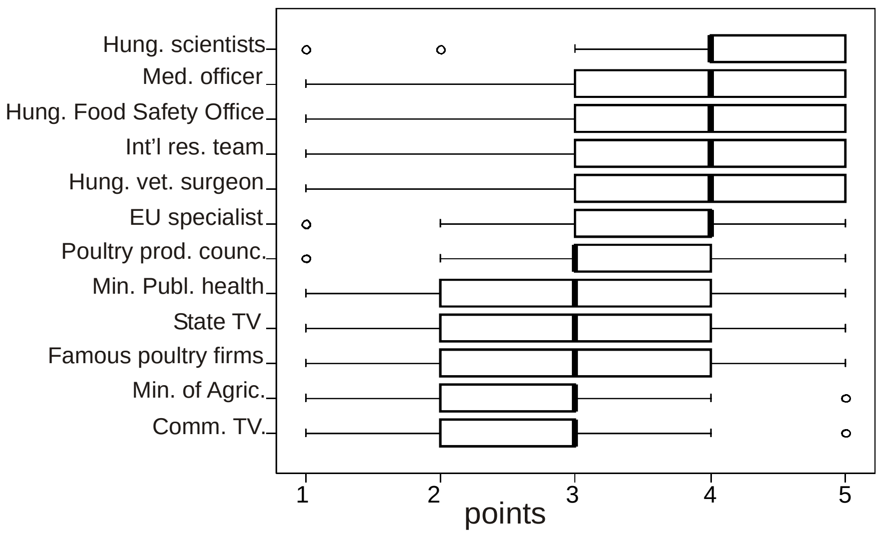


Figure 3

The level of trust in different poultry products

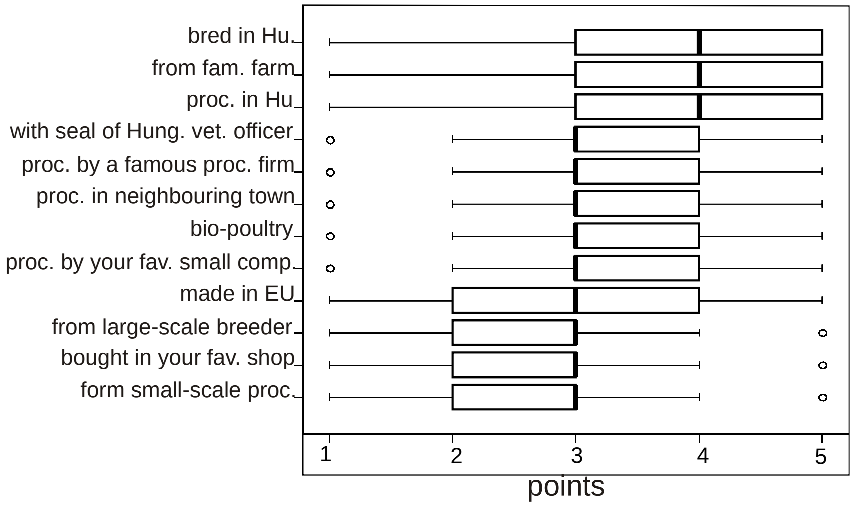
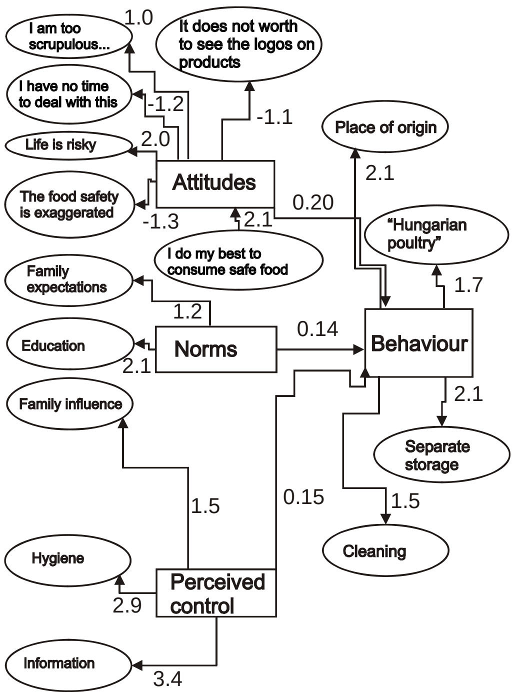


Figure 4

Results of structural equation modelling, for the determination of the consumer behaviour



Based on the data above, structural equation modelling was elaborated to determine the influence of different factors on the efficiency of a farm. The chi-square test showed that fitness of the model was significant, indicating that the null hypothesis, that the model fits the data, cannot be rejected. This finding was corroborated by the Root Mean Square Error of Approximation (RMSEA) statistics. According to *Muthén* (2000, 2004) the recommended cut-off value is 0.06. The RMSEA estimation was 0.1, that's why the model fits does not fits perfectly, but in our opinion there are important for practical purposes lessons. Values on the arrow indicate the regression coefficients.

CONCLUSIONS

The H_1 and H_2 hypotheses have been proven. The H_3 hypothesis has been proven only partially, because there is a rather high level of confidence for authorities. Mathematically, the Fishbein-Ajzen model (hypothesis No. H_4) does not fits perfectly to the data, but this can be a consequence of the relatively low number of answers. At the same time, the model is well-interpretable, the regression coefficients mirror the expected relations between the different measurable and latent variables. It would be challenging to determine the correlations between the variables "attitudes", "norms" and "perceived control" but this model has not been a significant one.

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SECTION 5

RABBIT BREEDING
AND
BEE-KEEPING

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Genetic parameters of production traits in Pannon White rabbit

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ABSTRACT

Genetic parameters for body weight at 5 (BW5) and 10 (BW10) weeks of age and average daily gain (ADG) from 5 to 10 weeks of age were estimated using univariate and bivariate animal models in Pannon White rabbits from 2001–2005. Of the influencing factors, age (BW5, BW10), sex (BW5, BW10, ADG) and year season (BW5, BW10, ADG) affected the examined traits. Heritabilities of BW5, BW10 and ADG were 0.21 (0.01), 0.26 (0.01) and 0.27 (0.01), respectively. Common litter effect of BW5, BW10 and ADG were 0.47 (0.01), 0.29 (0.01) and 0.20 (0.01), respectively. Genetic correlation between the body weight measured at different ages was 0.54 (0.05). Genetic correlation between ADG and BW5 and ADG and BW10 were -0.19 (0.06) and 0.76 (0.03), respectively.
(Keywords: genetic parameters, production traits, rabbits)

INTRODUCTION

Pannon White rabbit breed was developed at the University of Kaposvár. The selection programme consisted of several steps. Initially the dressing out percentage of the original New Zealand White population of the University was improved through progeny tests. Then Californian rabbits were used to cross the local New Zealand population to create a new synthetic line. Using the best crossing combinations the basis of the new line was created. The performance of this line did not exceed substantially the level of previous performance but the increased variance could be utilized in further selection. The synthetic line has been selected as a closed population since 1992. This line is recognised as the Pannon White rabbit breed. The population consists of cca 250 does and 60 bucks. The selection objective of the breed is to improve the average daily gain (between the 5–10th weeks of age) and dressing out percentage (which is indirectly estimated by means of Computerized Tomography). Detailed description of the breed's establishment and its selection programme is given by *Szendrő et al.* (1997). Evaluation of CT-based selection was published by *Szendrő et al*. (2004) and *Nagy et al.* (2006). The objective of the present study is to estimate genetic parameters of production traits of the Pannon White breed.

MATERIALS AND METHODS

The present analysis was based on data from 40 487 Pannon White rabbits born between 2001–2005. The evaluated animals were reared in 6 739 litters and the total number in the pedigree file was 42 578. The number of base animals was 269. Growing rabbits were housed in a closed rabbitry, in fattening cages (25×40×30 mm; 30×50×30 mm) (2–3 rabbits per cage, i.e. 20 rabbits per m^2). After weaning (35-d) they were fed a commercial pellet (16.3% crude protein, 15.2% crude fibre, and 10.6 MJ DE/kg). In winter the rabbit house was heated to a minimum temperature of 15–16 °C, while – in the absence of air conditioning – in the summer the temperature occasionally reached levels as high as 28 °C. Although in this study only the production traits were analysed the most important recorded traits are provided in *Table 1*. The number of measurements, means and standard deviations of the traits evaluated in this study can be seen in *Table 2*.

Table 1

Measured traits of the Pannon White rabbit breed

Reproduction traits	Production traits	Slaughter traits
- gestation length (day) - number of kits born total - number of kits born alive	- body weight measured at 5 weeks of age (kg) - body weight measured at 10 weeks of age (kg) - average daily gain measured between 5–10 weeks of age (g)	- slaughter weight (kg) - dressing out percentage (%) - cross sectional area of *m. longissimus dorsi* (cm^2) - weight of *m. longissimus dorsi* (g) - thigh muscle weight (g) - predicted (CT) thigh muscle weight (g) - perirenal fat weight (g)

Table 2

Number of measurements, mean and standard deviation of the evaluated traits

Traits	Number of records	Mean	Standard deviation
Body weight measured at 5 weeks of age (kg)	40487	0.89	0.18
Body weight measured at 10 weeks of age (kg)	40487	2.33	0.29
Average daily gain measured between 5–10 weeks of age	40487	41.19	6.93

The statistical analysis consisted of two consecutive steps. The first step was testing for the significance of fixed effects conducting least squares analyses using the GLM procedure of SAS (*SAS* Inc., 2005) leaving only significant factors in the model (*Table 3*.)

Table 3

Significance of the influencing factors of the examined traits

Factor	Type	BW5	BW10	ADG
Age (day)	C	*	*	-
Sex	F	*	*	*
Year-season	F	*	*	*

^{BW5}body weight measured at 5 weeks of age (kg); ^{BW10}body weight measured at 10 weeks of age (kg); ^{ADG}average daily gain measured between 5–10 weeks of age; ^{C}covariate; ^{F}fixed effect; $^{*}p<0.05$.

The second step was the estimation of the heritabilities of the individual traits and their genetic correlations. The method used to obtain the variance components was the animal model using the program PEST 3.1 (*Groeneveld*, 1990) (for data coding) and VCE 5 (*Kovac* and *Groeneveld*, 2003).

In case of BW5, BW10 and ADG the linear model was:

$$y = Xb + Za + Wc + e$$

where (according to *Mrode*, 2005): y= vector of observations, b= vector of fixed effects, f= vector of random effects, c= vector of common environmental effects (of the dam), X, Z, W incidence matrices relating records to fixed and random animal and random common environmental effects, respectively. Expected values of a, c, and e were E(a)= E(c) = E(e) = 0. The variance-covariance structure was assumed to be $V(a)= A\sigma^2_a$, $V(c) = I\sigma^2_c$, $V(e) = I\sigma^2_e$, and cov(a,e) = cov(e,a) =0, where A is the numerator relationship matrix. Also $cov(y,a) = ZAI\sigma^2_a$.

Regarding the model distribution of y was assumed normal, the traits were determined by many additive genes of infinitesimal effects at infinitely many unlinked loci. Due to the size of the data and the relatively low computing capacity heritabilities of the traits and genetic correlations between the traits were estimated using univariate and bivariate models, respectively.

RESULTS AND DISCUSSION

Heritability estimates of the analysed traits and the relative importance of the common environmental effects can be seen in *Table 4*.

Table 4

Relative importance of the additive genetic (h^2) and common environmental (c^2) effects (standard errors of estimates are given in brackets)

Traits	h^2 (SE)	c^2
Body weight measured at 5 weeks of age (kg)	0.21 (0.01)	0.47 (0.01)
Body weight measured at 10 weeks of age (kg)	0.26 (0.01)	0.29 (0.01)
Average daily gain measured between 5–10 weeks of age	0.27 (0.01)	0.20 (0.01)

Heritability of body weight measured at 5 week old rabbits was low. The heritability of body weight increased with the increasing age. Opposite tendency was received for common environmental effects that were decreased with the increasing age. Similar estimates were found by other authors (*Estany et al*., 1992; *Ferraz et al*., 1992; *Lukefahr et al*., 1996) justifying that the importance of maternal effects (i.e. mainly the milk production of the does) gradually diminish after weaning. *Szendrő et al.* (1988) also observed the decreasing heritability of body weight measured at ages of 6 and 10 weeks, however based on paternal half sibs they found much higher heritabilities (0.88±0.10; 0.66±0.10) than others. Heritability and common litter effects of average daily gain between 5 and 10 weeks of age were similar to that of the body weight measured at 10 weeks of age. Several authors received similar estimates for average daily gain (*Estany et al*., 1992; *Garreau et al*., 2000; *Krogmeier et al*., 1994) although *Moura et al.* (1997) reported higher heritability (0.48) and lower litter effects (0.11) than found in this study. The estimated genetic correlation coefficients among the examined traits are presented in *Table 5.*

Table 5

Genetic correlation estimates between the examined traits (standard errors of estimates are given in brackets)

Traits	r_g (SE)
BW5-BW10	0.54 (0.05)
BW5-ADG	-0.19 (0.06)
BW10-ADG	0.76 (0.03)

^{BW5}body weight measured at 5 weeks of age (kg); ^{BW10}body weight measured at 10 weeks of age (kg); ^{ADG}average daily gain measured between 5-10 weeks of age.

The estimated genetic correlation between the body weights measured at 5 and 10 weeks of age was only moderately high. Similar results were found by (*Lukefahr et al.*, 1996). Average daily gain showed high genetic correlation with the body weight of the latter age but the correlation was negligible with the body weight measured at the younger age. Similar conclusions can be drawn as in the preceding section. The body weight of the 5 week old rabbits was primarily determined by maternal effects and only secondarily by additive genetic effects. As the maternal influence gradually decrease after weaning the body weight of the 10 week old rabbits was mainly determined by additive genetic effects. Therefore the average daily gain between these ages was mainly independent of the 5 week old weight as it was primarily the performance of the doe rather than the growing rabbit. The latter body weight was however mainly determined by the rabbits' additive genetic effects and consequently it showed high genetic correlation with average daily gain. Previous genetic correlation estimate for this population was very similar (0.74) to the value received in this study (*Garreau et al.*, 2000).

CONCLUSIONS

Average daily gain between the ages of 5 and 10 weeks can be used as a selection objective. From the evaluated traits it had the highest heritability and lowest common environmental effect. Because of the high genetic correlation with the body weight measured at 10 weeks of age it can be expected that selection on average daily gain may reduce the maturity of the rabbits at slaughter if the slaughter weight is fixed. The maternal effects though decreasing with the increasing age they are still present at the age of 10 weeks. Therefore application of BLUP method in the selection can be recommended as it offers a better litter effect adjustment than other conventional methods.

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Comparison of oven drying with permeable film in substitution to freeze-drying in rabbit meat submitted to chemical analysis

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ABSTRACT

The study compared two meat drying methods, the classical freeze-drying and the oven drying with the introduction of the minced meat into bags made of permeable film of polyetherblockamide (Osmolux). Fourteen hindlegs from rabbits slaughtered at an age of 11 weeks, were deboned, minced and samples were either freeze-dried or oven-dried with Osmolux film. All of the samples were analysed for chemical composition, cholesterol content, and fatty acids (FA) profile. No statistical differences on the studied variables were found between the 2 drying methods. Determination coefficient R^2 *between the 2 drying methods was high for ash (0.95), dry matter (0.93), ether extract (0.90) contents, while for cholesterol content the* R^2 *was lower (0.74). Relationship between methods were weak for FA with* R^2 *values ranging from 0.57 for polyunsaturated FA, to 0.59 for saturated FA, to 0.69 for monounsaturated FA.*
(Keywords: rabbit, meat, chemical analysis, oven drying, permeable film)

INTRODUCTION

Freeze-drying technique is currently used to prepare meat samples prior to their chemical analysis, in order to reduce their water content, spoilage and the hydrolytic reactions. The limit of the freeze-drying is its high cost and the limited number of samples that can be prepared with each batch. The oven drying method is susceptible to waste minerals, vitamins and other meat constituents. Osmolux is a gas and vapour permeable film which is proposed for the storage and packaging of some vegetable products (*Moras* and *Lambert*, 1997). Its use for drying purpose could be economically advantageous, but no information are available on the chemical constituents preservation of the meat.

The aim of this study was to verify the effectiveness of oven drying with gas and vapour permeable films OSMOLUX (polyetherblockamide) in preparation of rabbit meat submitted for chemical analysis.

MATERIALS AND METHODS

Fourteen hindlegs from rabbits of 11 weeks of age were deboned. The meat was minced (Retsch grinder, 4000 r.p.m. per 12 sec) and split into two samples that were either freeze-dried (Control) or oven-dried with Osmolux (Permeable film). Osmolux is a gas and vapour permeable film of polyetherblockamide produced by P.A.T.I. S.p.A. A layer of meat about 0.7 cm thick was made using a roll bar up, and then placed into Osmolux bags, sealed and oven-dried at 60 °C until constant weight after 2 consecutive weightings.

After drying, all of the samples were ground (Girmi type FR 51 grinder, 20 sec) and stored at room temperature until analysis. Samples were analysed for chemical composition (DM, ether extract, ash) and protein was calculated by difference according to the standards of the *A.O.A.C.* (1984). Cholesterol content was also determined (*Casiraghi et al.*, 1994). Fatty acids (FA) profile was determined using a gas-chromatography, after Folch extraction, according to *A.O.A.C.* methods (1984). The chemical analyses were completed within a month from the drying.

Variance analysis was performed using the GLM procedure of the SAS program (*SAS Institute,* 1990) by including drying method as fixed effect. Determination coefficients R^2 , and regression equations between drying methods were calculated for all variables using Excel spreadsheet.

RESULTS AND DISCUSSION

The average chemical composition of rabbit's hindleg meat (*Table 1*) were not influenced by the drying method, except for the protein content on the as is basis ($P<0.05$). The protein content was calculated by difference, so all of the small differences emerged on the other analysed variables relapsed into the protein content.

Table 1

Chemical composition of hindleg meat

Samples, n.	Control	Permeable film	RSD	Probability
	14	14		
DM, %	25.9	26.1	1.5	ns
Protein, %DM	83.3	84.4	4.4	ns
Ash, %DM	4.5	4.5	0.4	ns
Ether Extract, %DM	12.2	11.1	4.7	ns
Cholesterol, mg/100g DM	239	250	33	ns
Moisture	74.1	73.9	1.5	ns
Protein, %	21.5	22.0	0.5	P<0.05
Ash, %	1.15	1.16	0.04	ns
Ether Extract, %	3.2	3.0	1.4	ns
Cholesterol, mg/100 g	61.3	64.8	6.1	ns

Comparing the fatty acid (FA) profile of the two groups (*Table 2*), it appeared that oven-drying with Osmolux permeable film, doesn't affected the compositions of fat. Differences were reported only for some of the less representatives FA, such as the C24:0, C24:1 n-9, C20:5 n-3 ($P<0.01$) and CLA (Conjugated Linoleic Acid, $P<0.05$). Saturated Fatty Acids (SFA) and Monounsaturated Fatty Acids (MUFA) values increased for permeable film group, while Polyunsaturated Fatty Acids (PUFA) decreased, but reported differences were not statistically significant. These small differences could be partly ascribed to the variability of the FA analysis and also to the FA hydrolysis which may have occurred on Osmolux samples during storage.

Table 2

Fatty Acid profile of hindleg meat

Samples	Control	Permeable film	RSD	Probability
	14	14		
SFA	37.5	40.5	6.4	ns
C6:0	0.16	0.38	0.32	ns
C10:0	0.12	0.13	0.07	ns
C12:0	0.14	0.16	0.05	ns
C14:0	1.93	2.11	0.55	ns
C16:0	25.0	27.1	4.5	ns
C18:0	7.8	8.1	1.2	ns
MUFA	26.0	27.7	4.0	ns
C14:1	0.10	0.11	0.09	ns
C16:1	2.02	2.17	1.16	ns
C17:1	0.45	0.46	0.04	ns
C18:1	22.4	24.0	2.8	ns
PUFA	32.6	28.8	10.7	ns
C18:2 n-6	27.8	24.9	9.4	ns
C18:3 n-3	1.67	1.27	0.77	ns
C20:4 n-6	1.67	1.17	0.98	ns

Determination coefficient R^2 between the 2 drying methods was high for ash (0.95, *Figure 3*), dry matter, DM (0.93, *Figure 1*), ether extract (0.90, *Figure 2*) contents, while for cholesterol content (*Figure 4*) the R^2 was lower (0.74).

Figure 1

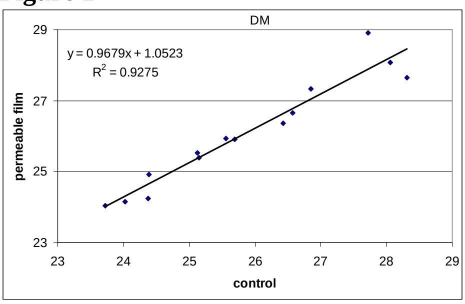


Figure 2

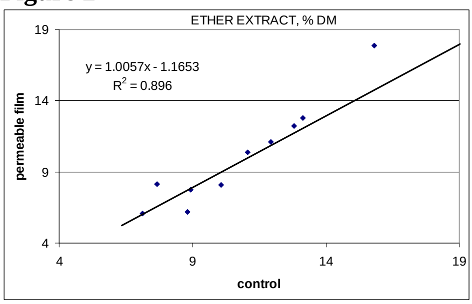


Figure 3

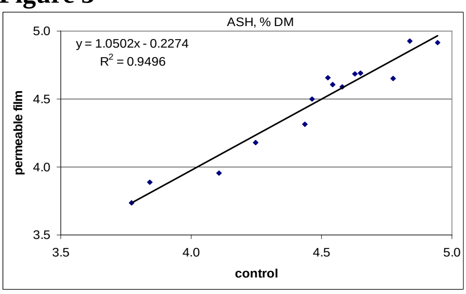
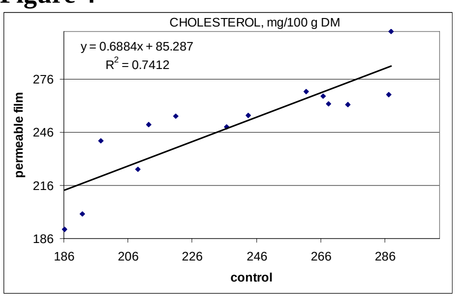


Figure 4



The R^2 values found on FA were worse than chemical analysis, ranging from 0.57 of PUFA (*Figure 7*), to 0.59 of SFA (*Figure 5*), to 0.69 of MUFA (*Figure 6*). Higher R^2 values were obtained for C10:0 (0.80), C12:0 (0.81), C14:0 (0.84), C16:1 (0.97) and C20:1 n-9 (0.80).

The present work aimed to approach the use of economical drying system, i.e. the oven drying with permeable films, and no particular attention was paid to the grinding methods and to the storage conditions. It is likely that the relationship between methods values could be improved by optimizing the grinding, before and after drying, and the storage conditions (time and temperature) of permeable film drying system.

Figure 5

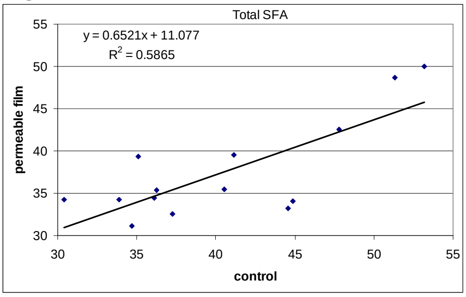


Figure 6

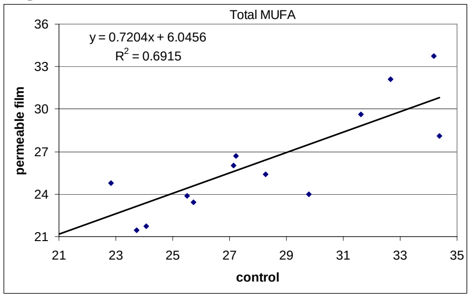
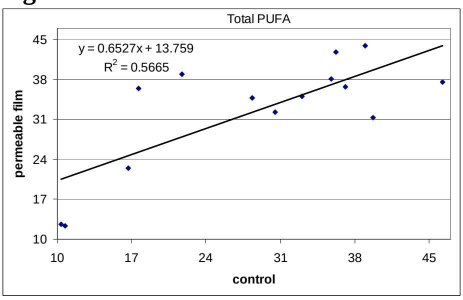


Figure 7



CONCLUSIONS

In conclusion, the oven drying with gas and vapour permeable films Osmolux did not affect the content of meat macro-constituents and its use could be suitable for screening purposes in rabbit meat. Further studies should aim at the improvement of this new method of water removal by testing the effects of grinding methods and storage conditions of the samples prior chemical analyses.

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The influence of different types of beehives (made of different material) and of the origin of Queens on the development of diseases

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ABSTRACT

Our investigation was aimed at determining the extent to which different types of beehives: Langstroth-Root (LR), Dadant-Blatt (DB) and Albert-Žnideršić (AB), made of different material (poplar-tree, lime-tree, fir-tree), with queens of different origin (natural and selected), influence the development of diseases in the hives. All bees used in the investigation belong to the European bee species Apis mellifera carnica. Research results indicate the interplay of all the factors (different types of hives, origin of Queens, and type of wood used for the hive) in the development of diseases in the hives.
(Keywords: beehive types, origin of Queens, diseases)

INTRODUCTION

In order to investigate the factors that influence the development of diseases in beehives, we decided to study the influence of different types of beehives made of different material, and using different queens.

MATERIALS AND METHODS

The research was conducted in Vukovarsko-srijemska County in the Republic of Croatia. During the investigation we used the following types of beehives: Langstroth-Root (LR, n=265), Albert-Žnideršič (AŽ, n=285) and Dadant-Blatt (DB, n=136). The wood used for the hives were poplar-tree (n=150), lime-tree (n=154) and fir-tree (n=382). We used natural (n=426) and selected queens (n=260).

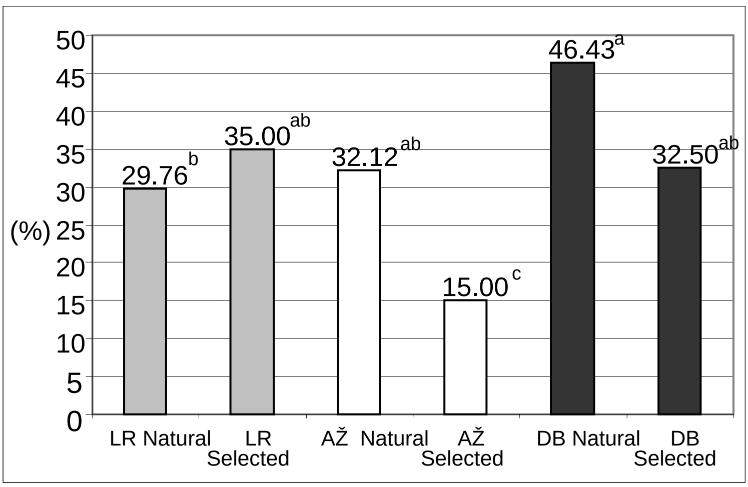
All bees used in the investigation belong to the European bee species *Apis mellifera carnica*. The honeybee brood obtained food and nutrients by visiting various species of the honey vegetables: Oil-seed Rape (*Brassica olespeciesa* subsp. *Oleifera*), False-acacia *(Robinia pseudacacia*), Lime tree (*Tilia* spp.) Horse-chestnut (*Aesculus hippocastanum*), Sunflower (*Helianthus annuus*), Goldenrod (*Solidago* spp.), Mint (*Mentha* spp.), False indigo (Amorpha fruticosa), Meadow Sage (Salvia pratensis) and other meadow plants.
The influence of different types of beehives, different material and types of Queens was determined using the attributive marker test. The differences between the groups ($P<0.05$) were marked using the letters a, b, c, and d. Research results were analysed using Statistica v7.1.30.0 (StatSoft.Inc 1984–2005).

RESULTS AND DISCUSSION

The influence of types of beehives on the development of diseases has been identified by *Tucak et al*. 2002 and 2003. *Figure 1* indicates the frequency of nosema in queens of different origin in different types of beehives. Most bees with nosema were found in DB type beehives with natural queens (46.43%). In LR type beehives with queens of both origins, in AŽ type beehives with natural and DB type beehives with selected queens, the incidence of nosema was about 30%. The incidence of nosema was the lowest in AŽ type beehives with selected queens (15%), which is statistically significantly less than in all other investigated groups. Statistically significant differences (P<0.05) were also found between DB beehives with natural queens, and LR beehives with natural queens, as well as AŽ beehives with selected queens. With AŽ and DB beehives we noted the tendency towards a higher incidence of nosema with natural queens compared to LR beehives. The highest incidence of nosema was found in AŽ beehives (32.89%), statistically significantly higher (P<0.05) than in DB beehives (24.91%). We have not found statistically significant differences (P>0.05) between LR and DB beehives regarding the incidence of nosema. In beehives with selected queens we have recorded a smaller share (P<0.05) of individuals with nosema (25%) compared to the beehives with natural queens (32.86%).

Figure 1

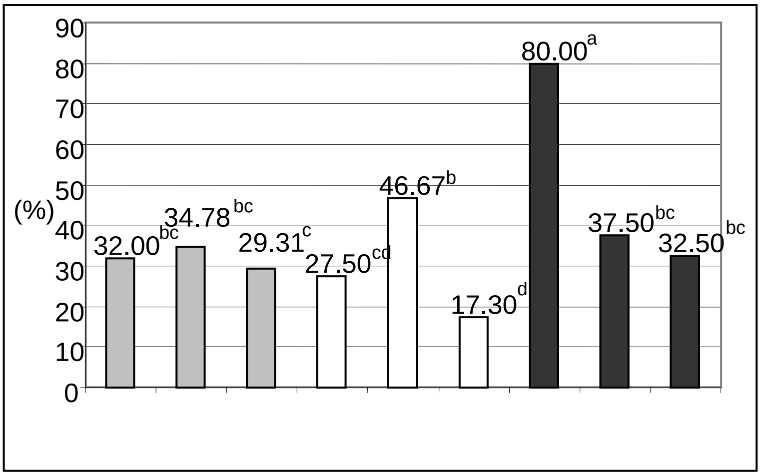
Incidence of nosema in queens of different origin in different types of beehives a, b, c P<0.05



The influence of different materials on the occurrence of nosema in the investigated beehives is shown in *Figure 2*. The highest incidence of nosema was found in DB beehives made of poplar-tree (80%), statistically significantly higher than in all other investigated groups. A significantly higher incidence of nosema (P<0.05) was found for AŽ beehives made of fir-tree compared to LR and AŽ beehives made of fir-tree and poplar-tree. AŽ beehives made of fir-tree had the lowest incidence of nosema compared to other groups. In all three types of beehives the smallest incidence of the disease was found for the ones made of fir-tree (29.31%, 17.30% and 32.50%). In LR and AŽ beehives the highest incidence of nosema was found in the ones made of lime-tree (34.78% and 46.67%), whereas in DB beehives it was the highest for the ones made of poplar-tree (80%). The lowest incidence on nosema was found for beehives made of fir-tree (24.15%), statistically significantly lower ($P<0.05$) than for those made of poplar-tree (34%) and lime-tree (40.26%). The differences in the incidence of nosema between beehives made of poplar-tree and lime-tree were not statistically significant ($P>0.05$).

Figure 2

Influence of different materials on the occurrence of nosema in LR, AŽ and DB beehives a, b, c, d P<0.05



CONCLUSION

- The highest incidence of nosema was found in AŽ beehives (32.89%), statistically significantly higher ($P<0.05$) than in DB beehives (24.91%). We have not found statistically significant differences ($P>0.05$) between LR and DB beehives regarding the incidence of nosema.
- In the beehives with selected queens we have recorded a lower incidence ($P<0.05$) of nosema (25%) compared to beehives with natural queens (32.86%).
- The lowest incidence of nosema was found for beehives made of fir-tree (24.15%), statistically significantly lower ($P<0.05$) than for beehives made of poplar-tree (34%) and lime-tree (40.26%).
- The highest incidence of nosema was found in DB beehives with natural queens (46.43%). The lowest incidence of the disease was found in AŽ beehives with selected queens (15%), which is statistically significantly lower than in all other investigated groups.
- In AŽ and DB beehives we have noticed a trend of the increasing incidence of nosema in beehives with natural queens compared to LR beehives.

- In DB beehives made of poplar-tree we have found the highest incidence of nosema (80%), statistically significantly higher than in all other investigated groups. AŽ beehives made of fir-tree had the lowest incidence of nosema compared to other groups.
- For all three beehive types, the incidence of nosema was the lowest for those made of fir-tree (29.31%, 17.30% and 32.50%).

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POSTER SECTION

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Animal production towards sustainable farm business management

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ABSTRACT

Farm management adjustment from centrally planned economy towards market-oriented farm development is difficult, especially in the Baranya region, since it is the agriculture, as the primary sector, on which other economic activities are based, both the secondary (manufacturing) and the tertiary (service trade) sectors. Therefore, agriculture is the way to the stability of national development in the economic integration of all sectors and the balance of development of the Republic of Croatia. Otherwise, tensions and conflicts might arise, which would directly influence the structural adjustment in farm production as the primary sector. A lack of economic resources and knowledge in farm management can be a major obstacle to sustainable development, respectively a dynamic farm production continually adjustable to both internal and external factors. Family farms have a special place in the said processes, because they are the most represented economic subjects in the primary farm production in the Baranya region. Since there is the interaction between animal production and crop growing, the relation between limiting and improving economic resources regarding regional economic development should be closely monitored.
(Keywords: animal production, sustainable farm management)

INTRODUCTION

The processes and activities in the period of transition with regard to the Croatian application for admission to the European Union influence the increase of competitiveness of animal produces at international markets, and in this way not only the sustainability of natural environment but also the farm management comes into question.

Sustainable growth and development of family farms is closely connected with the sustainable ways of animal production. Family farms managers have to do business economically efficiently, socially acceptable, competitively towards market and responsibly towards environment. Domestic animals represent a link between plants and the human, since animal production enables the human to produce produces of high quality, to speed capital turnover up and to stabilize both his income and business operations.

The terms such as "alternative", "ecological", "biological" or "organic" are used in science, practice and politics to define plant and animal produces that are contrary to the system of traditional or conventional farm production. It is unterstood that in cases where the new ways of production are applied, the dominant economic principles are as much as possible in accordance with ecological requirements.

The purpose of sustainable production management is to adjust dynamic economic development with ecological requirements of natural environment.

MATERIALS AND METHODS

Investigation of the basic assumptions in connection with the application of sustainable family farm business was done in 8 municipalities, respectively in totally 32 villages, so that the whole Baranya region was included in the investigation. The investigation was done in 271 family farms, and the data were collected by highly skilled interviewers, for which reason the gained information may be defined as relevant. Afterwards, the collected data were processed in terms of statistics. This investigation was done within frames of the "Programme for Sustainable Development of Family Farms in Baranya", financed by EU CARDS Project – Contract No. 99754. In the paper the methodology according to *Krstić* (2000) was applied, according to which the number of conditional head of cattle was calculated on the basis of the following formula:

$$Z = \frac{U}{P}$$

Z = number of conditional head of cattle per hectare of farmland,
U = number of physical head of cattle,
P = farmland in hectares.

RESULTS AND DISSUSSION

Animal production is not only directly but also indirectly connected to crop growing. On the one hand, direct connection derives from farmlands used for fodder production; in this way the continuity of both animal feedstuffs and the supply safety of production process in animal production is preserved. On the other, indirect connection is very important in the system of sustainable management of economic resources on the farm. Production of stable litter and manure, as by-products in crop growing and animal production, provide sustainability of farmland and have a special ecological importance in relation to industrially produced mineral fertilizer. Although modern industrial methods are sometimes used in specialized crop growing and animal production, beside higher costs of production, they also lead towards farmland destruction and pollution of the environment, which is not in any case the purpose of sustainable management of family farms. Hence, it is important to monitor the size as well as property structure of farmland per family farm.

Table 1

Arable land and property structure of family farms in Baranya

No.	Municipality	Land size per family farm (ha)	Private land (%)	Land tenure (%)	Leased out land (%)	Average number of parcels per farm	Adjustment to ecological production (%)
1	Bilje	25.47	61.25	38.75	0	12	13.85
2	Čeminac	36.10	57.79	42.21	0	8	11.54
3	Darda	*14.38*	34.63	64.66	0.71	4	2.04
4	Draž	*73.69*	38.54	61.46	0	10	15.38
5	Jagodnjak	30.13	36.18	63.82	0	8	0
6	Kneževi Vinogradi	48.61	49.48	50.52	0	13	27.27
7	Petlovac	37.56	46.63	53.37	0	11	27.30
8	Popovac	22.32	38.40	61.60	0	10	3.13
Average		**36.03**	**45.36**	**54.55**	**0.71**	**9.5**	**12.56**

Source: investigation done by the authors.

Average arable land per family farm is 36.03 ha (*Table 1*), respectively this number is significantly above national average of 2.9 ha. The size of property varies from municipality to municipality (14.38–73.69 ha) and it results in the possibility to determine what the farmland will be intended for: large farmlands will be used in crop growing (industrial plants), whereas smaller ones will be used in animal production, vegetable crops, fruit or grape growing, or grpwing of other cultures, for which intensive production large farmlands are nor required.

Relation between private land and land tenure, is 45:55%. Not only this data confirms a lack of private arable lands, but it also shows that population has chosen farming for their primary and existential job, since more than a half of arable land has been taken out on a lease from both legal and natural persons.

A large number of small plots of land is a limiting factor in sustainable development, because it increases costs and time necessary to do all required technical and technological measures, which directly influences efficiency and productivity. There is a significant number of family farms oriented towards to ecological production, especially those with bigger arable lands.

Table 2

Property structure according to the land size (%)

No.	Municipality	1–4.99 ha	5–9.99 ha	More than 10 ha	Total
1	Bilje	37.50	26.56	35.94	100.00
2	Čeminac	18.52	22.22	59.26	100.00
3	Darda	30.61	22.45	46.94	100.00
4	Draž	7.14	21.43	71.43	100.00
5	Jagodnjak	7.69	7.69	84.62	100.00
6	Kneževi Vinogradi	21.21	6.06	72.73	100.00
7	Petlovac	4.55	9.09	86.36	100.00
8	Popovac	15.63	21.88	62.50	100.00
Average		**17.86**	**17.17**	**64.97**	**100.00**

Source: investigation done by the authors.

The analysis of property structure according to the land size per family farm shows dominance of farms with more than 10 ha farmland, respectively 64.97% (*Table 2*). Smaller farms (up to 5 ha) and medium-size farms (from 5−10 ha) are almost equal in percentage terms, respectively 35.03%. These types of family farms have to adjust their farm management to diversified production orientation, which gives competitive advantage to animal production. (*Clakins*, 1983).

The rate of animal production is expressed by a number of conditional head of cattle per farm, respectively per hectare of farmland, in order to establish the number of cattle per production unit or per area. Conditional head of cattle is a physical indicator, respectively an accounting unit, a result obtained by multiplication of the number of physical head of cattle with coefficients used as a main criterion to place on the same level all breeds and categories of cattle. As the basis a head of cattle of 500 kg body weight is used and it is represented by a coefficient 1.00. All other breeds and cattle categories are amounted to with regard to average weight of the category (*Krstić*, 2000).

Table 3

Animal production – structure of breeds and categories of conditional head of cattle*

No.	Breed and category of cattle	Coefficient	Bilje	Čeminac	Darda	Draž	Jagodnjak	Kneževi Vinogradi	Petlovac	Popovac	Total
1	Bovine	1.00	41.00	40.00	21.00	85.00	135.00	80.00	90.00	107.00	599.00
2	Calf	0.25	0.75	3.00		5.50	6.50	6.75	15.25	5.50	43.25
3	Fattening cattle	0.50		12.50		29.00	1.50	42.50	12.50		98.00
4	Horse	1.00		4.00		1.00	2.00	28.00		3.00	38.00
5	Sow	0.30	40.20	22.80	16.50	3.60	17.40	10.80	10.20	24.90	146.40
6	Hog	0.30	0.60			0.30	0.60		0.90	1.80	4.20
7	Porker	0.25	162.30	59.50	111.00	23.75	29.75	57.00	43.00	35.75	522.05
8	Hen	0.002	2.11	0.84	1.70	0.35	0.88	1.45	0.68	1.57	9.58
9	Broiler	0.00055	0.42	0.06	0.27	0.08		0.19	0.09	0.08	1.19
10	Turkey	0.01	0.43	0.69				0.09	0.11	0.12	1.44
11	Duck	0.006	0.57	0.15		0.12		0.04	0.16		1.04
12	Goose	0.01	0.52	0.28		1.46			0.26		2.52
13	Rabbit	0.002	0.08	0.13	0.13			0.07	0.69		1.10
14	Sheep and ram	0.10			23.60	14.40	80.00	21.70	45.00	83.50	268.2
15	Lamb and tag	0.05		33.30	10.55	8.20	28.00	3.80	10.00	0.75	94.6
16	F/M goat	0.10	5.40	10.55	1.60	7.00	2.20	2.10	35.80	0.30	64.95
17	Baby goat	0.05	0.55	0.20	1.10	6.25			4.75		12.85
18	Donkey	1.00							1.00		1.00
19	Ostrich	0.25							8.75		8.75
Conditional head of cattle totally			**254.93**	**188.00**	**187.46**	**186.01**	**303.83**	**254.49**	**279.14**	**264.27**	**1918.12**

Source: investigation done by the authors.
*UG - conditional head of cattle - head of cattle of average body weight (500 kg).

When animal production was analysed and the number of conditional head of cattle was calculated, a number of conditional head of cattle was also calculated in municipalities that had different number of farms. some of which were not oriented towards animal production (*Table 3*). Therefore, the relationship between the total number of conditional head of cattle and the number of animal production oriented farms was established. In this way, indicators regarding intensity of animal production per farm in different municipalities were obtained. When it comes to analysis of the number of conditional head of cattle in the relation to crop growing in hectares of land per farm, it is possible to establish the intensity of farm production per municipality. Moreover, it is posssible to determine unknown resources either for the development of animal production or for primary crop growing for the needs of animal production and manufacturing.

Number of conditional head of cattle per hectare is a significant indicator of the stage of development and intensity of farm production (*Table 4*). In order to evaluate the presence of animal production. there are 6 levels of intensity:

- Very high: 1 : 1
- High: 0.8 : 1
- Medium: 0.5–0.6 : 1
- Low: 0.4–0.5 : 1
- Weak: 0.25–0.4 : 1
- Very weak up to: 0.25 : 1

Table 4

Regional sustainability of animal production development in numbers (conditional head of cattle)

Description	Bilje	Čeminac	Darda	Draž	Jagodnjak	Kneževi Vinogradi	Petlovac	Popovac
Number of animal production oriented farms	50	24	36	14	20	26	22	33
Total number of conditional head of cattle	254.93	188.00	187.46	186.01	303.83	254.49	279.14	264.27
Conditional head of cattle per farm	5.10	7.83	5.21	13.29	15.19	9.79	12.69	8.01
Hectares/farms	8.53	9.91	17.44	12.06	27.19	6.96	35.14	7.20
Conditional head of cattle/ hectare	**0.60**	**0.80**	**0.30**	**1.10**	**0.56**	**1.41**	**0.36**	**1.30**

According to the data shown in *Table 4*. there are three municipalities characterized with a very high level of intensity of animal production (37.5% of the investigated region), two municipalities with a medium level of intensity (25%), whereas in the remaining two municipalities the level of intensity is weak (25%).

When compared to the national average (0.5 UG/ha), this relationship is favourable because of specific features and possibilities of the Baranya region: economic resources, soil capacity and nearness of unexploited manufacturing capacities. The fact that animal production is insufficiently present in the region shows that despite available possibilities (the changes in animal production and crop growing), the production has not been intensified (UG/ha).

These possibilities should be taken in consideration when preparing measures for future improvement of animal production. Farm managing is one of the factors that leads towards sustainability, but also a means to improve economic efficiency in production and business. This should be realized. if one wants to achieve sustainability and dynamics in business and farm managing.

CONCLUSION

Long-term sustainable development should be directed towards the growth of land. In this way animal production would be influenced directly. Animal production is the basis of liquidity and economic stability, because of equalizing the possibilities both to gain income and to pay obligations. It influences added value and improves the value of crop growing by closing the manufacturing cycle important for ecological sustainability of production and business on the farm. The results show that there are many possibilities to develop animal production, since 50% of the investigated region is characterized by a medium to low level of intensity of animal production. A lack of animal production reveals up to now unknown economic resources in the Baranya region. If activated, it would be possible to obtain sustainable farm management in natural environment.

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Total quality management – penetration presumption on world market of animal products

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ABSTRACT

Animal production in the Republic of Croatia is in unfavorable position considering loss of traditional market and demand decrease on domestic market what affects unused production factors. Such situation requires creating of essential conditions and presumptions for growth of animal production export, regarding terms of strong global competition and technological development. Adoptions of quality and security systems are factors and priorities of competitiveness. It presents important role in reaching aims considering importance of international competition in introducing of global quality management. Quality management and security system of animal products presents system management approach for increasing values for consumers by creating and constant improvement of organization processes and systems. Introducing of HACCP system and good production practices as well as ISO 9001 are complex international standards related to specification of management system and process. Processors should adopt those standards in order to insure constant quality improvement as presumption for distribution of animal product to European and world market. Therefore, producers and processors in Republic of Croatia regarding quality management and security of animal products are at the beginning of this process, what requires more intensive and responsible adopting of international standards.
(Keywords: global competition, international standards, TQM, quality costs)

INTRODUCTION

Entering 21st century is reflected in higher globalization of world market on the one hand, and on the other in faster development of higher technologies. Croatian agrarian structure is connected with terms of tough business condition and realistic possibilities. Croatian agriculture has a task of strategic direction for creating short-term profit as well as terms for more stabile and successful long-term development. Agrarian structure of transition countries barely started to deal with those business conditions due to loss of previous markets and decreasing demand on present domestic market. Necessary reforms for sustainable economic development in Croatia are not applied completely, so the transformation to modern competitive economy is required considering international business and global competition. One of the most important presumptions is approach to quality, which was connected to product at the beginning and lately it presents processed approach to quality and methods of Total Quality Management (TQM). It is in line with FAO and WTO requirements considering reforming of global food technology systems through modern technologies and increasing of food trade. Those great changes will provide improving of nutrition and sufficient amount of biologically valuable animal products. Complete fulfilling of international norms means only establishing necessary and good basic for gradually achieving of success business. Success business today is the aim of leading producers of animal products who must provide all requests of capital owners, buyers, suppliers, employees and social community. It is possible to achieve with assistance system and constant improving of higher complete quality. Constantly improving of total quality requires effective management connected with business issues and necessity of TQM. Systematic and efficient development of TQM is possible to achieve by applying business success model regarding establish of balanced business entities effect. Modern and adaptable system of modern management must provide fulfilling of ISO norm connected with management system, selected model of business success and self evaluation as well as adopting of prevented measures of self control (complex HACCP system).

MATERIALS AND METHODS

Managing animal product quality is group of all firm's activities in order to provide buyers demands regarding quality and norms. Correctly created and applied ISO system for quality providing and management brings internal and external direct and indirect gains (*Injac*, 2002). Implementing of quality system is important for firms searching position on international market. Therefore, in the paper will be presented results of researching conducted in the Republic of Croatia on representative sample of firms implementing ISO norms and HACCPS system. Following criteria of stratifications are used: firm size, activities and regions. Collected date were analyzed according to SPSS and compared to similar results of *Bešker* (2001), *Sikavica* and *Bahtijarevic-Šiber* (2004), *Drljača* (2003).

RESULTS AND DISCUSSION

Animal production represents strategic branch of improving and development of agriculture production. It is very important considering position in value structure of agriculture wealth and total national wealth. Productivity system of animal products must be adopted according to changeable environment providing quality system applying and market performances.

Table 1

Changes of animal production from 1986 to 2005 in the Republic of Croatia

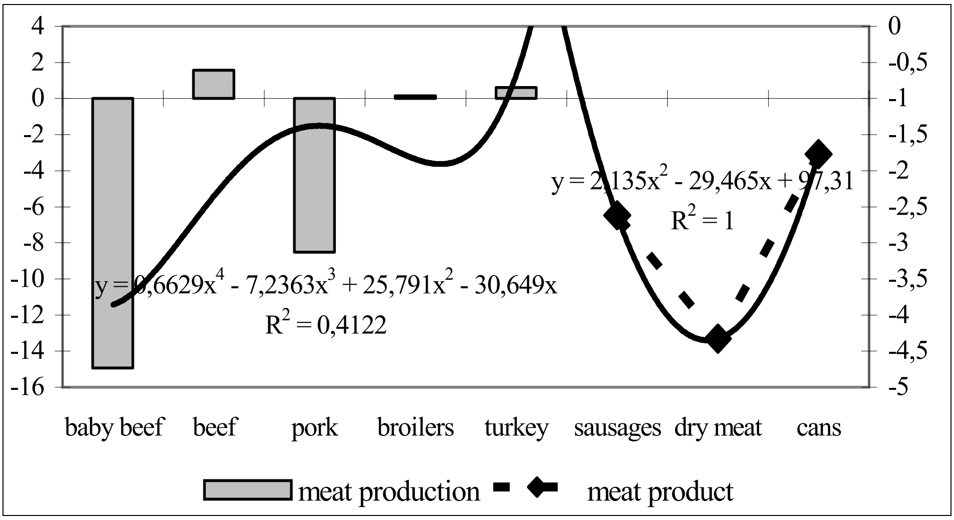
Products		Average value		Variation Interval		Parameters	
		X_{min}	X_{max}	s.d	R^2	F	Annual rates
Growth (000) tones							
Beef	90,27	54	144	33,17	0,82	60,35	-5,05
Pork	202,87	163	271	40,87	0,68	28,27	-2,55
Sheep	8,73	5	13	2,60	0,26	4,55	-0,75
Poultry	98,07	67	120	18,96	0,28	5,00	-0,76
Product u (000)							
Milk (l)	748,87	588	1013	171,2	0,77	45,33	-3,17
Wool (t)	536,87	351	764	151,2	0,71	31,13	-3,16
Eggs (piece)	914,33	804	1096	109,4	0,78	46,47	-1,74
Honey (t)	947,8	627	1398	236,2	0,60	19,40	3,39

Source: SLJH, 1993 and 2003: 223., 240.

Total livestock growth in 2005 was 1.5 times lower compared to 1986. Average annual decreasing rate of livestock growth volume was 2.62%. The highest growth decreasing was noticed for beef (average decrease rate 5.05%) and lowest for sheep (0.75%). The lowest average annual decreasing was registered for eggs production (1.74%) whence honeys production was the only positive production volume (annual rate 3.39%). However, production of final animal products was constantly increasing as a result of increasing of domestic demand as well as export possibilities. Furthermore, the final livestock production was increased by average rate of 1.42% as a lower growth compared to total final agriculture (1.7%) and plant production (1.57%). Meat production, especially beef meat has the highest decrease (47.36%) and average annual rate was 14.93% as a consequence of forbidden export of live beef (foot disease, BSE) and pork (rate -8.51%). This trend is result of war (decrease of fertile animals), enormous import, weak export and decreased consumption of mentioned meats. Positive trend (from 0.6 to 6.5%) was noticed for beef, poultry and turkey meat were per average annual rate from 0.14 to 1.58%.

Figure 1

Meat production and meat products from 1995 to 2005



Production of animal product shows increasing trend by annual rate from 1.77 to 2.62%. Reasons causing this trend are decreased domestic animal production, low protection of domestic production (tariffs and quotas) as well as insufficiently defined direction of TQM development and adoption in slaughtering meat -processing industry. It caused high import of meat and products. During globalization, firms involved in animal production are faced with unfavorable position of tough competition, because demanding conditions cause strategy of stable and successful long-term development. Therefore, creating of condition towards higher quality and labor productivity, cost decreasing and export increasing is very important because only simultaneous orientation on consumers and concurrent insure permanent market success. European Union adopts quality systems according to international ISO standards involving TQM principles. Arranged standards represent documents, which set up business systems and provide higher quality level. Aims of introducing quality systems are:

- Completely setting of business systems and subsystems,
- Profit increasing,
- Cost decreasing,
- Achieving higher level of product quality,
- Providing quality management from idea to post-consumption,
- Penetration of business system to world market,
- Possibilities of increasing sell price according to quality,
- Decreasing of cost price caused by decreased quality costs,
- Accomplishing market demands.

ISO norms in the certificate form insure permanent approach of animal production to international market, what is very important for Croatian firms. In fact, Croatia tries to adopt fact that process to accomplish successful firm business is via quality development and system quality. Numerous Croatian firms are in standard preparing phase, while only 792 firms in 2005 had certificates, meaning process of introducing model and quality system is on the beginning.

Table 2

Number of certificates according to ISO norms in Croatia

Years	Certificates (N)
1996.	11
1997.	17
1998.	21
1999.	33
2000.	35
2001.	74
2002.	97
2003.	152
2004.	195
2005.	157

The greatest certificate share is related to biggest Croatian firms are exposed to competition for gaining better position on international market. Dynamical increase of certificates it following years can be expected considering Croatian commitments regarding accessing European Union. Research results in Croatia in 2000 and 2001 (*Drljača*, 2003), show connection between business success and quality what affects:

- Increase firms with certificate,
- Share of firms with certificate among the most successful exporters is higher than among the most successful importers,
- Significance and role of standardization and quality is higher in production compared to unproduction economy branches.

206 firms were analyzed in order to define attitude of producers and processors of animal products about adoption of managing quality system according to ISO norms. 56 firms or 27% from total sample adopted ISO quality system and 14% takes consultations regarding quality norms.

Table 3

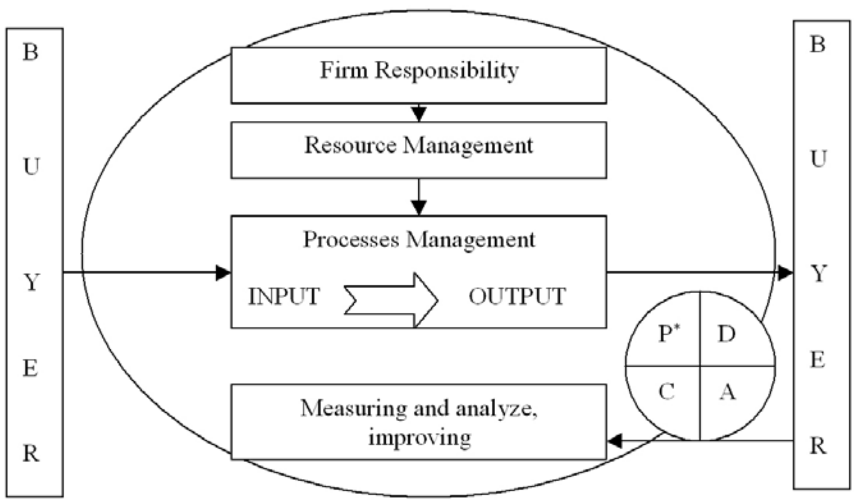
Structure firms according to implementation ISO norms

	Number	Structure	ISO certificates	ISO consultations
Processing industry	183	89 %	27 %	14 %
Agriculture and forestry	19	9 %	6 %	2 %
Fishery	4	2 %	6 %	4 %

4% agricultural firms confirmed positive attitude in introducing ISO norms and certificates because 22% conduct preparations and 32% take consultations. Economic activities were grouped according to χ^2 test about independences of criteria and hypotheses about firm dependence were accepted regarding applying quality systems. Based on results, 65% with certificates are from industrial activities and 49% has positive attitude concerning introducing quality system. With aim of more success penetration to world market mentioned firms must adopt TQM. Takes in consideration responsibilities of all processes in system: management of resources and processes, measurement and improvement analyze. Thereby all mentioned processes are connected to vertical and closed model improving TQM structure.

Figure 2

Total Quality Model in Animal Production



* P - plan, D - do, C - check, A - act

All these activities were conducted aiming customer's expectations fulfilling. Measuring, analyze and improving is important in order to bring feedback to management confirming and approving changes and taking improving actions. Horizontal line in model represents significant of consumer role regarding input data processes, sets requirements and feedback as an important element for permanent business improvement.

CONCLUSION

New experiences and requirements are directed towards quality system, which must be developed in order to insure product quality involving all function and all employees. Quality insurance needs to be achieved via connected suppliers and producers. Modern customer’s request is high quality and improved product. All mentioned above leads to conclude that Croatian firms involved in production and processing of animal products need to adopt following principles:

- Modern market is consumer market not producer market including all consequences and new requests.
- Product quality is in the interaction with other two factors. Price and supply dates as well as quality show extremely non-elasticity regarding market adoption.
- The fundamental goal of animal production should be customer satisfaction, for without satisfied customer, there is no long-term business. To achieve customer satisfaction requires going beyond customer expectations and providing products that never disappoint the customer.
- Term of partnership in modern business is fulfilling universal request concerning quality according to international norms. Knowing importance of transformation regarding higher phases of quality improvement, defining quality politics as well as graduallity and permanence is crucial for all activities in Croatian economy in order to join development world.

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The using of IFCN'S method in Hungary

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ABSTRACT

The idea of the International Farm Comparison Network (IFCN) was born in Braunschweig, Germany at the Federal Agricultural Research Centre (FAL) in 1997. The program was developed on the basis of the objective to create a unique database updated ever year that makes it possible to compare the economic charachteristics of typical farms operating world wide. The three main goals of IFCN are the following: - to establish and operate a world-wide network dealing with the analysis of agricultural systems, - to analyse and predict the effects of political and technological changes in the participating countries, - to promote contact and change of data between theory and practice. Within the IFCN the basis of the analysis is a simulation model called TIPI-CAL. TIPI-CAL is a recursive-dynamic, production-simulating and accounting model which was developed for farm analysis With the help of the model we can analyse the effects of the change regarding political measures and legal circumstances, which is shown mainly in different management strategies. In relation to this within the European Union adjustment strategies induced by different development processes are of special importance. These processes are analysed both on national and international level thus with individual simulation we can also have some idea about international competitiveness.
(Keywords: model, method, comparison, network)

INTERNATIONAL FARM COMPARISON NETWORK

The idea of the International Farm Comparison Network (IFCN) was born in Braunschweig, Germany at the Federal Agricultural Research Centre (FAL) in 1997. The principles were developed together with the Agriculture and Food Policy Centre (AFPC) of the A&M University, Texas which had been doing research in this field for 15 years and had established an international system of typical plants extending it to Mexico and Canada.

Due to globalization the comparative advantages of the regions have more and more importance, and these can modify the circumstances of agricultural production. The basic question – which product should be produced by which production region – has an effect not only on the political decision-makers but also on people working in the agribusiness and on farmers as well. The questions related to this issue are the following:

- Is there an estimate to show the effects of alternative liberalization strategies on different types of farms?
- What are the main reasons for the lack of competitiveness?
- What is the best national strategy to enhance the competitiveness of a country?
- What are the effects of different systems on the environment at different points of the world?

Until the extension of the IFCN system these questions could not be answered due to the lack of information source that could have given an overall picture regarding these issues within a reasonable time. The reasons for this are the following:

- The majority of the agro-economists concentrate on the macro-economic level. The studies dealing with the competitiveness of a country were based on sources of information that were no longer valid at the time of the publication. Due to the different research methods the applicability of the data to compare the results can be questioned. In addition to this several studies put emphasis on the past instead of dealing with objective analysis about the future.
- The majority of the models used internationally deal only with the surface of the problem.However, it is possible to make a comparison between plants in different countries, this level is not thoroughly examined and there are a lot of questions to be answered. One of them refers to the possibilities of development in a region with regard to competitiveness.

As a consequence IFCN has three main goals:

- to establish and operate a world-wide network dealing with the analysis of agricultural systems,
- to analyse and predict the effects of political and technological changes in the participating countries,
- to promote contact and change of data between theory and practice.

Due to the development of IFCN there are three different sections with researchers from more than 35 countries (dairy production 33 countries, crops 13 countries, beef and sheep 15 countries). The Faculty of Economic Sciences of the University of Kaposvár has been participating in the network from the beginning.

THE METHOD OF IFCN

TIPI-CAL is suitable for simulating real and so-called typical plants as well. With view to political counselling it is more adequate to deal with the latter and determine the general political conditions and production process of a given region.

A typical farm is developed with the participation of consultants, experts and managers of similar plants. Presumably a plant totally similar to this does not exist, however, the data fall into a track in which the production units of the region operate. This method has the following advantages compared with the arithmetical average of the accounting data:

- up to date data (the majority of the data of statistics are at least a year ago);
- individual phenomena can be eliminated;
- "mixed" farms made up of statistical data are usually not realistic.

Panel meeting consists of several steps:

- choosing the region;
- determining the size of the farm typical of the region (consulting with a local expert);
- the first collection of data by using a questionnaire filled in by the expert;
- the first panel when the correctness of the farm (compiled by the local expert) is discussed by 3–5 farm manager working in the same environment;
- the first simulations using TIPI-CAL;

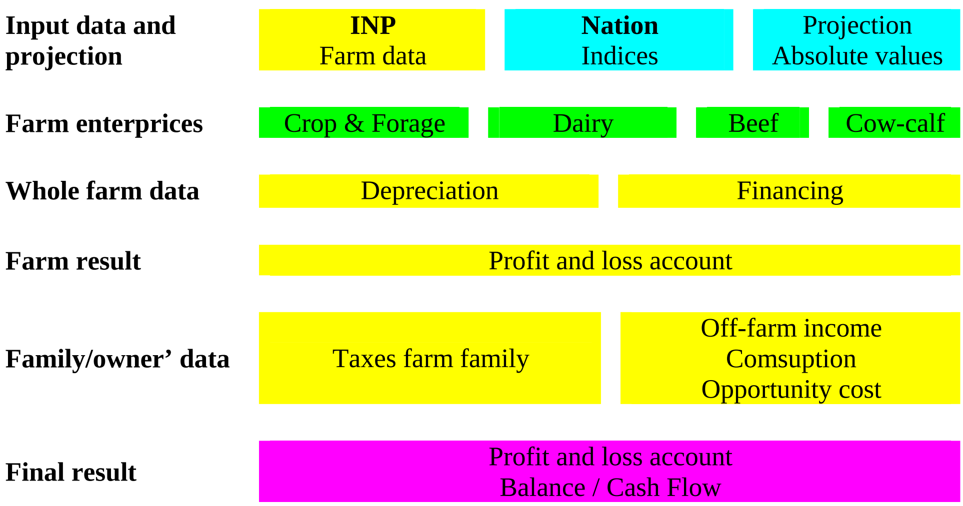
– the second panel: discussion of the correctness of the farm on the basis of the first model results, making corrections if necessary;
– closing the typical farm, carrying out further simulations.

At this point it has to be mentioned that the operation of the above mentioned "classic panel" is difficult in Hungary. Compiling the farm is predominantly the task of the user, experts help only in special fields. A panel meeting with 3–5 producers is not feasible because it would require 10 people (two experts from each farm providing breeding and accounting data). In order to solve this problem the discussion takes place at the farm with the experts.

TIPI-CAL is a recursive, dynamic, production-simulating and accounting model for plant modelling developed by the Farm Economics Institute of the Federal Agricultural Research Centre (FAL) of Germany in collaboration with the Agriculture and Food Policy Centre (AFPC) of the Texas A&M University (*Figure 1*). The model was developed in 1997 particularly for analysing milk production plants. This model type is primarily suitable for beef finishing and cow-calf analysis was developed in 2001.

Figure 1

Content of TIPI-CAL (tm3-beef.xls)



Source: www.ifcnnetwork.org

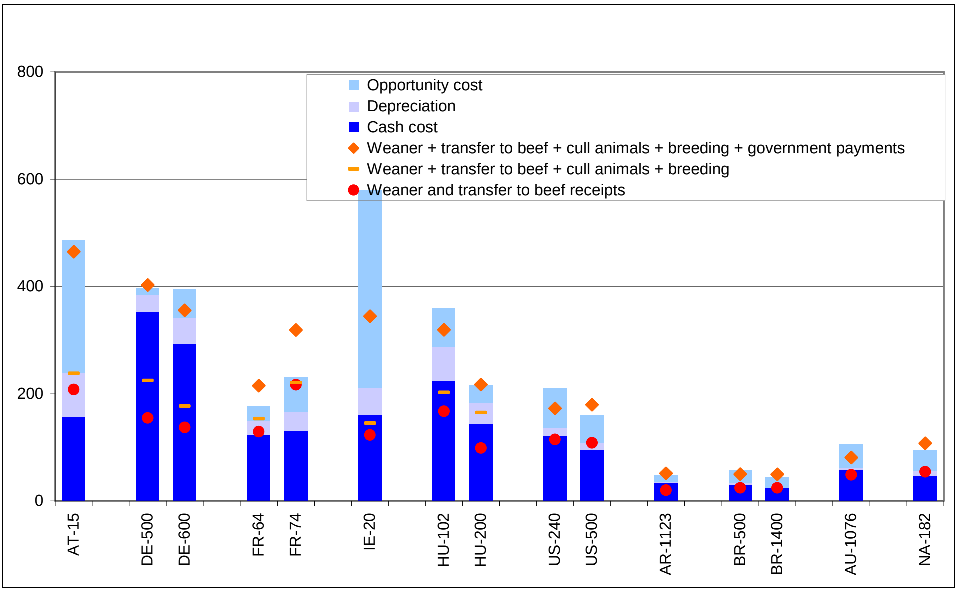
The model demonstrates the effects of political measures and changes in the legal environment on farming, especially the ones determining different management strategies.

The model is basically a simulation of the agricultural production. At the end of each economic year it makes a balance, profit and loss account and Cash Flow and it is also possible to determine profit-sharing and after-tax profit. Though the model has an internal controlling system – especially as regards allowances in kind – the correctness of the plant data and expense price rate has a considerable influence on the result. The model input consists of two parts: first farm data and strategies then prices and yield are processed. Of course, it is possible to adapt the model to the general conditions of the economic policy of the country. The model has a "matrix" as a complementary element for the analysis of different economic policies, management and organization strategies. It is possible to analyse and compare 10 different environments of economic policy, and farm management strategies.

Figure 2. shows the costs and returns of the cow-calf farms. It shows that the returns of the Hungarian plants, just like those in the majority of the European countries cover only a part of the opportunity costs. In case of the Hungarian plants the revenues from the *weaners* do not even cover the cash cost. Basically these plants are dependent on state subsidy. Southern-American and Australian plants have an exceptionally low level of costs which is particularly due to the extensive range management.

Figure 2

Total returns and cost by cash and non-cash cost

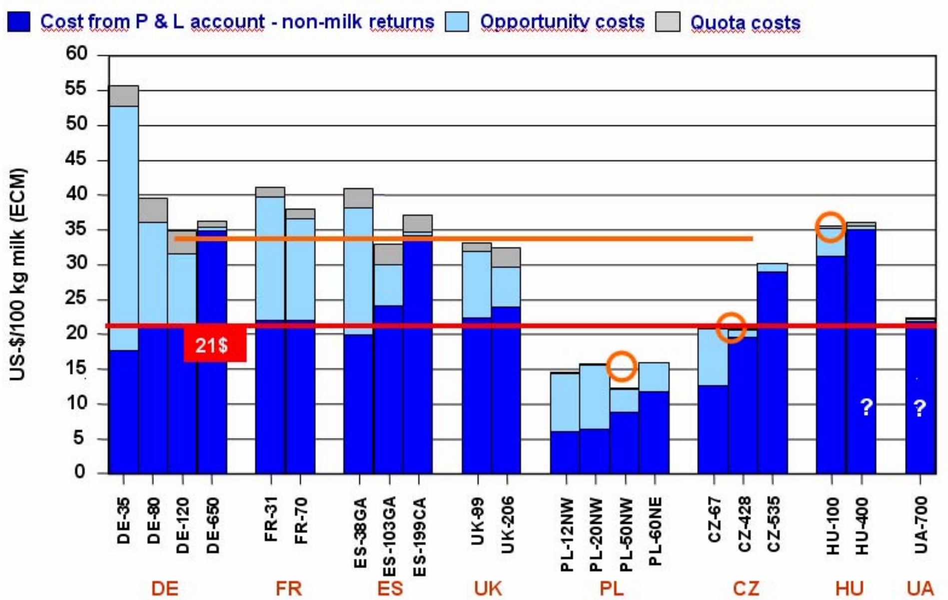


Source: IFCN Beef Report 2003

Figure 3 shows the total cost of production in the examined countries. It reflects the fairly high cost of production. a characteristic of the Hungarian milk production in the past few years. In Hungary this is primarily due to the high forage costs but cost of supplement for livestock and labour costs are also determining factors. Surprisingly the former cooperative farm in East-Germany and the Spanish 199 farm – said to be large under local conditions – are considered to be similar to the Hungarian 400 farm. The Polish plants are classic family farms operating at low costs, however, their efficiency and productivity are far beyond those of the competitors.

Figure 3

Total cost of production



Source: IFCN Dairy Report 2005

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Novel methods of Fusarium toxins' production for toxicological experiments

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ABSTRACT

Studies were performed to develop a novel, efficient and cost-effective method for fumonisin and T-2 toxin production, respectively, in sufficient quantities for animal toxicological experiments. On the basis of three earlier published fumonisin toxin production methods a novel method was developed. Three, absolutely necessary factors were taken into account and tested in a serial experiment. The Fusarium verticillioides strain MRC 826 was directly inoculated onto soaked, autoclaved, whole maize kernels (50 g/1.7 l jar). The inoculation was performed by standard spore suspension ($1 \cdot 10^6$ /ml), a 5/2 surface/volume culture was prepared and incubated at 25 °C for 5 weeks. To maintain the optimal a_w of approximately 1.00, the evaporated water was re-filled weekly. A final concentration of 4454±1060.9 mg kg^{-1} fumonisin B_1 was reached. T-2 toxin was produced experimentally on ground maize by Fusarium sporotrichioides strain NRRL 3299. With the method in this study similarly high T-2 toxin concentration can be reached as in case of fumonisin production. In the laboratory practice, applying the above settings, a high toxin production can be obtained.
(Keywords: fumonisin B_1, T-2 toxin, production methods)

INTRODUCTION

Fumonisins, a family of food-borne carcinogenic mycotoxins, were first isolated in 1988 (*Gelderblom et al*., 1988) from cultures of *Fusarium verticillioides* (Sacc.) Nirenberg (previously known as *Fusarium moniliforme* Sheldon). Fumonisin B_1 (FB_1) typically accounts for 70 to 80% of the total fumonisins produced, fumonisin B_2 usually makes up 15 to 25% and fumonisin B_3 usually from 3 to 8%, when cultured on corn or rice. (*Marín et al.,* 1999). FB_1 is responsible for several toxicological actions in animals. These include the neurotoxic syndrome, leukoencephalomalacia in horses, pulmonary edema in pigs, and hepatocarcinogenic and hepatotoxic effects in rats (cit. *Marasas et al*., 1993). FB_1 is carcinogenic and has been implicated in the pathogenesis of oesophageal cancer in humans (*Marasas et al*., 1993).

Although cereals are important substrates, moisture level and temperature are the critical abiotic factors regulating the growth of *F. verticillioides* and the production of fumonisins (*Cahagnier et al.,* 1995). The best temperature range is 20–28 °C for fumonisin production (*Alberts et al.,* 1990) but low kernel moisture content (less than 22%) should reduce or prevent toxin production during storage (*Le Bars et al*., 1994). *Marín et al*. (1995) surveyed the effects of different temperature and water activity (a_w; the water available for fungal growth) values on fungal growth and fumonisin production by *F. verticillioides* and *F. proliferatum* strains, observing that both increased when the moisture and temperature increased. Since fumonisins have been known only for a short time, adequate information concerning their toxicology is lacking.

In the method of *Fazekas* (1998), the toxin was produced by *F. verticillioides* strain 14/A as inoculum, grown on maize. Since then the production ability of this strain was first decreased, and later discontinued, probably due to the successive inoculations. (*Fazekas*, 2004). Other methods (*Vismer et al.*, 2004; *Alberts et al.*, 1990) were previously tested in our laboratory, but little quantities of FB_1 were reached (*Fodor*, 2005). In this respect, the need to develop efficient methods for the production of fumonisin toxins in sufficient quantities is essential.

Table 1

Comparison of the reference data

Concerning reference		**Methods**		
		Fazekas, 1998	***Alberts et al.,1990***	***Vismer et al., 2004***
Strain		14/A	MRC 826	MRC 826
Growing the strain on any agar		On Czapek agar for a week and on new Czapek agar slants once again for the same duration	-	-
Inoculum		Unknown spore number	Unknown spore number	Standardized spore suspension ($1 \cdot 10^6$ spores per ml)
Medium		100 g of whole yellow corn kernels and 70 ml of water	400 g of whole yellow corn kernels and 400 ml of water	30 g ground yellow maize kernels and 30 ml water
Preparation of medium	Soaking	overnight	1 h	1 h
	Autoclaving	for 1 h at 121 °C and 120 kPa on each of two consecutive days	for 1 h at 121 °C and 120 kPa on each of two consecutive days	for 1 h at 121 °C and 120 kPa on each of two consecutive days
	Means of FB_1 production	2-liter glass fruit jars with a cover, in a closed room	2-liter glass fruit jars with a cover, in incubator	18 cm diameter petri-dishes
Conditions of production	Temperature	25 °C	25 °C	25 °C
	Light	darkness	darkness	darkness
	Duration	4 weeks	11 weeks	14 days
	Shaking	In the first week daily	No information	-

The vast majority of trichothecenes are produced by the Fusarium species. Production of these metabolites depends, of course, on many factors, including substrate, temperature, humidity, etc. In general, the type A trichothecenes have been most frequently associated with the following *Fusarium* species: *F. tricinctum, F. sporotrichioides, F. poe, F. equiseti.*

Interest in these compounds stems from their occurrence as contaminants in major crops and their acute toxicity in humans and domesticated animals upon consumption (*Marasas et al.*, 1984). Many of the cytotoxic characteristics of the trichothecenes are attributed to their ability to inhibit protein synthesis (*McLaughlin et al.*, 1977) and to induce apoptosis in eukaryotic cells (*Okumwai et al.*, 1999). Various methods for the production of trichothecene mycotoxins have been reported (*Burmeister*, 1971; *Bata et* *al.,* 1984) but none of them are satisfactory for mass production for the great number of animal experiments. Because of deficiencies the database, even in the quantification of NOEL (No Observed Effect Level), only a temporary TDI (Tolerable Daily Intakes) could be determined for trichothecenes (*Schlatter*, 2004). Thus, further longer-term animal studies are clearly justified with in which a NOEL would be identified.

The need to develop efficient and cost-effective methods for the production of fumonisin and trichothecene toxins in sufficient quantities for a variety of biological studies is essential, since amounts of these mycotoxins produced under natural conditions are far too low for this purpose. There are only few data available on the reproducible experimental conditions for the optimal production of these toxins in culture. The objective of the present study was to describe a novel, alternative method for fumonisin and T-2 production, respectively.

MATERIALS AND METHODS

The strain of *F. verticillioides* used in experiments was originally isolated from corn in Transkei, southern Africa and deposited in the culture collection of the South African Medical Research Council (MRC) as *F. verticillioides* MRC 826 (*Marasas et al*., 1984). Based on three different, earlier published methods (*Table 1*), a novel method was worked out. During this process three, absolutely necessary factors were taken into account and tested in a serial experiment, as follows: 1.Quantity of medium: 50 g; 2. Standardized spore suspension from the lyophilized conidia: $1{\cdot}10^6$/ml; 3. Water added weekly to flask: 10 ml.

The method developed based on the factors tested is the next: Maize was prepared in 1.7-liter wide-mouthed glass fruit jars (diameter: 11 cm) with a (cotton plug between 2 linen rags) cover by autoclaving 50 g yellow maize kernels in 50 ml water (>50% moisture content; ≈1.00 a_w) for 1 h at 121 °C on two consecutive days, after an overnight soaking. The prepared maize was inoculated with a standard spore suspension. The number of spores in the freeze-dried vial was counted (with making tenfold dilutions until the 10^{10} dilution), and adjusted to $1{\cdot}10^6$ spores per ml. Whole corn cultures (surface/volume ratio, approximately 5:2) were directly inoculated with 1 ml of the spore suspension. The flasks were closed, and shaken manually to homogenize the culture material. The inoculated cultures were placed in a pre-sterilized incubator (LP-122, Labor-MIM, Hungary) to avoid contamination of the cultures and were incubated at 25 °C for 5 weeks, in air-flow function. To prevent a stuffy condition and possible fermentation, furthermore to get a better fungus distribution on the medium, all jars were vigorously shaken daily for 7–8 days. According to our preliminary investigations, in order to keep the proper water activity (a_w 0.98–0.93; *Cahagnier et al*., 1995), 10 ml sterile distilled water was added to each flask weekly.

T-2 toxin was produced experimentally on corn grits by *Fusarium sporotrichioides* strain NRRL 3299 (Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL), as described below.

Maize was prepared in 4.2-liter wide-mouthed glass fruit jars with a cover by autoclaving 800 g yellow corn grits (size: 2–3 mm) for 2 h at 121 °C, after an overnight soaking and filtering process. The inoculum was produced by growing the fungus on Czapek agar for 8 days at 25 °C. Spore suspensions were prepared by adding 2·5 ml of sterile, distilled water to the sporulated cultures. After dislodging of the conidia by gently scraping the agar surface with a sterile inoculation loop, the suspension was transferred into sterile, autoclaved maize. The culture was incubated in darkness at 24 °C for a week, then at 8 °C for 2 weeks. After opening, the fungus-infected maize was dried at room temperature for some days, then ground. The resulting meal was stored at 4 °C until it was used in chemical analysis.

All treatments and culture methods were performed in triplicate (i.e. three cultures were homogenized together), and each experiment was repeated three (FB_1) and six (T-2) times, respectively. Determination of the actual fumonisin toxin concentration of the samples was carried out by a HPLC fluorescence detection method (LOD 0.05 mg kg^{-1}) based upon pre-column derivatisation with *ortho*-phthalyldialdehyde (*Fazekas*, 1998), while T-2 mycotocin was determined with GC-MS (LOD: 0.01 mg kg^{-1}).

RESULTS AND DISCUSSION

Fumonisin production

The experimental series are illustrated in *Table 2*. According to the three, previously published methods (see *Table 1*), the optimum constant incubation temperature for FB_1 production was identical, i.e. 25 °C, therefore this parameter was not altered. The use of ground kernels for the production was omitted, since in our pre-experiments with maize patties, the culture became stuffy, namely the necessary air amount for the mycelium growth was not met in the interior of the culture. Taking our experiences into considerations, the medium quantity (Factor 1) in the methods of *Alberts et al*. (1990) and *Fazekas* (1998) seemed to be far too high, accordingly, this parameter was reduced to 50 g, so the resulting surface/volume ratio strongly exceeded those in the published methods. It is also necessary to inoculate the corn cultures with a standardized spore suspension (Factor 2), otherwise the production of fumonisins will be negatively influenced. If the freeze-dried vials' contents are prepared from carnation leave agar (CLA) cultures, the same, i.e. standardized inoculum can be used each time. In order to reach almost equal production per culture, cultures were inoculated with standard spore suspension, since without this criteria, the standard deviation of the toxin concentration per jar was very large. Fumonisin biosynthesis by *F. verticillioides* is very much dependent on a_w. A 10% decrease in $a_{w,}$ i.e., from 1 to 0.90, reduced the quantity of FB_1 produced 300-fold (*Nelson et al.,* 1991). Based on this fact, in order to avoid water vapour elimination from the jars, sterile water was continuously dosed (Factor 3). *LeBars et al.* (1994) found that the total FB_1 concentration per batch after 3 weeks was lower than that after 2 weeks; this reduction was attributed to the decrease of oxygen during incubation. Namely, the fungus may also break down its own toxin after producing it. Therefore, in order to avoid an oxygen deprived status in the jars and the consequent decrease of fumonisin content, in our experiment the incubation period was 5 weeks. Applying all the above mentioned factors (Factor 1, 2 and 3), high production was reached. The toxin production started on the first week (*Figure 1*) and continued to increase after this phase, contrasting with the results reported previously (*Alberts et al.,* 1990), i.e. that the production commenced at 2 week. The mean and the relative standard deviation of the results obtained were 4454.6 mg kg^{-1} FB_1 and ±1060.9, respectively.

T-2 production

The first stage in the present study was to find a satisfactory strain for T-2 production. As shown in *Table 3*, the strain is able to produce T-2 toxin on corn. In comparison with the conditions of FB_1 production, the *F. sporotrichioides* strain is relatively non-sensitive to medium quantity (800 g). The corn grits were inoculated with a spore suspension of the fungus prepared on Czapek agar. The count of spores was unknown.

Table 2

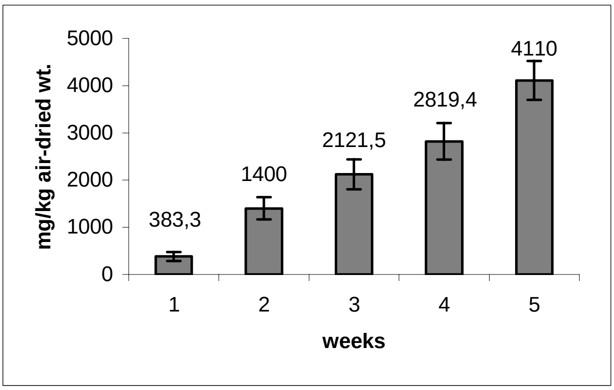
FB_1 produced in the experimental series for the development of the novel method

Factors applied*	Factor 1	Factor 1 and 2	Factor 1,2 and 3
FB1 Content of triplicate jars ($mg\ kg^{-1}$ air-dried wt.)	621.4	1600	4110
	942.1	2185.7	3609
	611.8	1059.2	5645
Mean	725.1	1614.9	4454.6
±S.D.	±187.9	±563.3	±1060.9

*Factor 1.Quantity of medium: 50 g; Factor 2. Standardized spore suspension from the lyophilized conidia: $1 \cdot 10^6$/ml; Factor 3. Water added weekly to flask: 10 ml.

Figure 1

Time course of FB_1 production by the strain MRC 826, taking all factors into consideration (means of triplicate jars; n=3)



T-2 toxin production by Fusarium spp. has been evaluated previously on liquid (*Ueno et al*., 1975) and solid media, including autoclaved cereal grains (*Burmeister*, 1971). However, these methods have been criticized for lengthy, low-temperature incubations required (*Cullen et al*., 1982), poor yields and inordinate amount of incubator space required (*Richardson et al.,* 1984). Growing the fungi on a liquid medium required a shorther incubation period, but yields of T-2 toxin were low and variable, and the method required greater space in the incubator. Rice cultures of known toxigenic isolates grown yielded oily extracts containing compounds which interfered with qualitative and quantitative analysis for the toxin.

As compared with prior methods, our procedure resulted high and consistent amounts of T-2, moreover relatively rapidly. Therefore, the results suggested that this method has advantages over the others already reported.

According to our preliminary work, the a_w seems to be influential in toxin production because a great increase in the moisture content resulted much higher toxin concentration (5.87 g kg^{-1} T-2) in the cultures.

Thus, further studies are needed to investigate those potential factors which may cause a significant increase in the toxin production.

Table 3

T-2 toxin production by *Fusarium sporotrichioides* strain NRRL 3299

T-2 content of triplicate jars (mg kg^{-1} air-dried wt.)	591.5
	548.2
	706.0
	731.3
	531.9
	1180.4
Mean	714.8
S.D.	242.2

CONCLUSIONS

Infection of maize with *Fusarium* species and its contamination by different mycotoxins are generally influenced by many factors including environmental conditions (temperature, humidity) and pre-and postharvest handling. These factors do not influence infection independently but most often there are complex interactions. The higher concentration of nutrients and the loss of consistency due to the temperature treatment may enable the moulds to colonize the corn easily. Furthermore, the moisture conditions during the growing season as well as during storage are often pointed out to affect maize infection by *Fusarium spp*. and mycotoxins synthesis. In this context, water activity plays a key role. A possible reason for this lag is that the morphology and physiological functions of the fungi are dependent on a_w, and changes in a_w affect the ability of the fungi to produce mycotoxins because mycelial growth (at a_w 0.90) is less mycotoxigenic than sporodoquia (at a_w 0.98) production (*Nelson et al*., 1991). Moreover, from the viewpoint of toxin production, the number of infective spores and the type and availability of nutrients for fungus is also necessary. It is unfortunately a fact that not all batches of culture produce equally well, but with the following of standardized method, the production should not differ too much. In the laboratory practice, taking the above mentioned facts into consideration, a high toxin production can be reached both in fumonisin and T-2 production.

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Biomarker in the detection of fumonisin toxicosis in pigs

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ABSTRACT

The sphinganine to sphingosine ratio (Sa/So) after oral administration of 50 mg fumonisin B_1/animal/day for 10 days and after a 10-day elimination period was studied in weaned barrows. At the beginning of the experiment, then after a 10-day period of the toxin feeding and on the 10^{th} day after the end of the toxin feeding blood samples were taken and from those the Sa/So ratio was determined. Directly after the 10-day period of the toxin feeding the Sa/So ratio was 6.2-fold higher, as compared to control group and it decreased only in a slight *extent even after the 10-day elimination period. Based on the results, presumably the restitution in the Sa-So parameters is more slower than the elimination of fumonisin from the organism.*
(Keywords: sphinganine, sphingosine, fumonisin, biomarker, pig)

INTRODUCTION

Fumonisins are a family of mycotoxins that were first isolated in South Africa in 1988 from cultures of *Fusarium verticillioides* (*Gelderblom et al*., 1988), followed soon thereafter by elucidation of the structures of the prevalent isoforms fumonisin B_1 (FB_1) and B_2 (FB_2) (*Bezuidenhout et al.* 1988). Leukoencephalomalacia in horses and pulmonary edema syndrome in pigs (Ross et al. 1990) were shown to be result of FB_1 administration (*Kellerman et al*., 1990; *Harrison et al.,* 1990), and field outbreaks were associated with fumonisin contamination (*Plattner et al*., 1990) when analytical methods were developed (*Shephard et al*., 1990). Fumonisins were also implicated in esophageal cancer when they were found in home-grown maize in a high-incidence area of the Transkei region of South Africa (*Sydenham et al*., 1990). FB_1 has been demonstrated to cause liver and kidney cancer in rats and mice (*Gelderblom et al*., 1991), and the International Agency for Research on Cancer evaluated FB_1 as a Group 2B carcinogen, i.e., possibly carcinogenic to humans (*IARC*, 1993).

The structural similarity between sphinganine and fumonisin B_1 led to the hypothesis that this mycotoxin acts by disrupting the metabolism and/or function of sphingolipids (*Merrill et al.,* 1996). There is considerable support for the hypothesis that fumonisin-induced disruption of sphingolipid metabolism is an important event in the cascade of events leading to altered cell growth, differentiation, and cell injury observed both in vitro and in vivo (WHO, 2002). Complete inhibition of ceramide synthetase by fumonisins causes a rapid increase in the intracellular concentration of sphinganine and sometimes of sphingosine, both in vivo and in vitro (*Delongchamp* and *Young*, 2001; *Enongene et al*., 2000).

Numerous studies are available concerning the use of sphinganine to sphingosine ratio (Sa/So) in plasma during fumonisin exposure (*Riley et al*., 1993). In horses and pigs, it appears predictive of fumonisin exposure. By contrast, in other studies conducted in pigs, even if Sa/So also increases during exposure to the toxin, hepatic biochemical parameters appears more sensitive (*Rotter et al.,* 1996). Similar results were also reported in certain studies conducted in rodents (*Castegnaro et al*., 1996). In avian populations, both Sa/So and bio-chemical parameters of hepatic damage increase during fumonisin exposure (*Bermudez et al*., 1995).

The aim of the study was to get data on Sa/So ratio after a high FB_1 exposure, to get information about the effect of FB_1 on the mentioned biomarker of fumonisin toxicosis. Moreover, to get information on the degree of its regression after the withdrawal of the toxin containing diet.

MATERIALS AND METHODS

Experimental animals, design of the experiment

Weaned castrated pigs of approximately 12–14 kg body weight were used. Animals (treated n=10; control n=6) were placed in metabolic cages during the trial. A *Fusarium verticillioides* fungal culture was mixed into the feed of the experimental animals, so as to provide a daily FB_1 intake of 50 mg/animal, i.e. 3.5–4 mg/kg body weight/day.

The experimentally produced fungal culture contained a high amount of FB_1; this was mixed in a pre-defined proportion to the feed reaching the aimed toxin concentration. This was performed according to the modified method of *Fazekas* (1998). At the beginning of the experiment (day 0), than after a 10-day period of the toxin feeding (day 10) and on the10th day after the end of the toxin feeding (day 20), blood samples were taken, and the Sa/So ratio was determined.

The experimental procedure, the determination of the Sa/So ratio were performed at the University of Kaposvár Faculty of Animal Science. The trial is approved by the Animal Experimentation Ethics Committee of the University of Kaposvár.

Analysis

Analytical method for the determination of sphinganine sphingosine ratio was according to *Castegnaro et al*. (1998) after some modifications, as described below.

Equipment

An HPLC liquid chromatographic system (Shimadzu-Gynkotek) was coupled with manual injector port with 50 µl sample loop, a flourescence detector (Shimadzu RF551) and a data computing system (Chromeleon 4.30). Separation of sphingolipids was on a Supelco RP-Amyde C16 column maintained at room temperature.

Sphingolipid extraction

KCl (1.5 ml, 0.8%) and KOH (50 µl, 1 M) were added to 500 µl of serum. The mixture was extracted with 5 ml of ethyl acetate by gentle agitation with vertical shaker for 20 min. The phases were separated by centrifugation 600 g, 4 min. 4 ml of organic phase was evaporated to complete dryness at 50 ^{o}C under N_2.

O-phtalaldehyde (OPA) derivatisation

The derivatisation mixture consisted of 5 mg OPA in 1 ml of MeOH containing 8 µl of 2-mercaptoethanol and 5 ml of 3% boric acid solution (pH 10.5 with 1 M KOH). Following the ethyl acetate extraction the dried samples were dissolved in 100 µl of methanol-water mixture 9:1 by vortex shaking for 30 sec and derivatisated for at least 30 min of the above 50 µl OPA mixture.

HPLC analysis of the derivates
The derivates were analysed by HPLC with fluorescence detection (ex.: 340 nm em.: 455 nm). The column (Supelco RP Amide-C16 150x4mm, 5 µm) was kept at room temperature and the isocratic eluent (MeOH-water 84:16) flowrate maintained at 1 ml/min. The injection volume was 50 µl of derivated samples or standards.

Statistical analysis
Statistical analysis of the data obtained was carried out by the SPSS statistical software package using the version 7.5. All basic data (means, standard deviations, extreme values) were evaluated. Data were analyzed with one-way analysis of variance (ANOVA) and the least significant difference (LSD) test was used to compare means. Probability levels less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

It can be generally established that the fumonisin exposure in pigs caused a marked elevation in the concentration of free sphinganine, and to a lesser extent in sphingosine, with a consequent increase in the sphinganine to sphingosine ratio (*Table 1*). These findings show good agreement with the results obtained in the experiment of *Gumprecht et al*. (1998) on swine.

The sphinganine to sphingosine ratio was significantly increased on the last day of the toxin feeding (day 10), as a result of the toxin dose applied. The sphinganine to sphingosine ratio of fumonisin-treated pigs' plasma was significantly greater than those of control pigs' plasma, and than of those samples taken on day 0 (*Table 1*).

Table 1

Influence of oral administration of 50 mg fumonisin B_1/animal/day on the ratio of sphinganine to sphingosine (Sa/So) in plasma

Date	Sa/So (mean ± S.D.)	
	Control group (n=6)	Treated group (n=10)
Day 0	$0.43^{a} \pm 0.04$	$0.40^{a} \pm 0.10$
Day 10	$0.46^{a} \pm 0.03$	$2.85^{b} \pm 0.48$
Day 20	$0.42^{a} \pm 0.06$	$2.33^{c} \pm 0.21$

^{abc}Means with different superscripts are different at the 1% probability level.

Directly after the 10-day period of the toxin feeding the Sa/So ratio was 6.2-fold higher, as compared to control group. This parameter decreased only in a slight extent, i.e. even after the 10-day elimination period it was 5.5-fold higher than at the beginning of the experiment.

Fumonisin residues accumulated in liver and kidney when pigs were fed [^{14}C] FB_1 at dietary concentrations of 2–3 mg kg^{-1} for 24 days (*Prelusky et al*., 1996). When this was followed by clean food, residue levels dropped to about 35% of peak values after 3 days, and were only marginally above the detectable limits after 9 days. Sphingoid bases increased in serum earlier (within 24 h) (*Gumprecht et al*., 1998) at even much lower doses of fumonisin (≤5 mg kg^{-1}) (*Riley et al.,* 1993) than liver enzymes and before morphologic tissue alterations, suggesting that the serum concentration of sphingoid bases could be used as a sensitive biomarker of fumonisin exposure. However, the role of altered sphingolipid biosynthesis in the pathogenesis of fumonisin toxicity is still under investigation. There are no data available about the total restitution concerning the parameters of sphinganine to sphingosine ratio. Based on the results of the present study and the earlier published data about the elimination of FB_1 (*Prelusky et al.*, 1996), presumably the restitution in the Sa-So parameters is slower than the elimination of fumonisin from the organism. Therefore, to clarify entirely the elimination of FB_1 from the organism, including following up of the Sa/So ratio, further animal studies are needed with long-term elimination period.

CONCLUSION

The ratio of sphinganine to sphingosine increases after exposure to fumonisins; these increases occurred long before any indication of cytotoxicity (*Riley et al.*, 1994). The results of the in vitro studies suggested that changes in the relative amounts (i.e. in the ratio) of free sphinganine and free sphingosine might be useful as a biomarker for fumonisin consumption in animals (*Riley et al.*, 1994). Based on the results of the present study and the earlier published data about the elimination of FB_1 (*Prelusky et al.*, 1996), the restitution of the Sa-So parameters to the physiological values would presumably be slower than the elimination of fumonisin from the organism.

The determination of Sa/So alteration kinetics would give us the possibility to verify the consumption of fumonisin contaminated diet even when the toxin is already not detectable in it. Therefore, the objective of our future studies will be to determine the Sa/So ratio as a presumptive test for identifying animals that consumed a fumonisin-contaminated feed.

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Effect of atrophic rhinitis on the behaviour of piglets (preliminary results)

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ABSTRACT

In this study the influence of atrophic rhinitis on the behaviour of pigs was investigated. In the experiment piglets were weaned from their dam at the age of one day and were reared artificially under controlled animal house conditions. The behaviour of a group of uninfected control pigs was compared with those infected experimentally with Pasteurella multocida. The examined behaviour forms were feeding, drinking, resting, social behaviour, scratches and playing. On day 8 after P. multocida infection we observed that the piglets spent less time with eating and social activities than the control piglets while the time they spent with resting increased. Our examination gives solid evidence that reduced activity coincides with reduced time spent with feeding by the animals.
(Keywords: pig, atrophic rhinitis, behaviour)

INTRODUCTION

Atrophic rhinitis (AR) of swine is a long-known and widely prevalent infectious disease of pig populations, characterised mainly by twisting and shortening of the nose. Toxin-producing strains of *Pasteurella multocida* cause damage after colonising the nasal passages. Intranasal challenge of pigs with *P. multocida* can artificially induce AR (*Magyar et al.*, 2002). The damage caused pain (*Diemen et al.*, 1995) and/or the presence of cytokines (IL-1, IL-6) induced by acute inflammation may decrease the feed consumption (*Klasing* and *Johnstone*, 1991*; Langhans*, 2000).

The role of the aerial conditions, management factors and hygiene on AR was investigated by others (*Penny*, 1977; *Robertson*, 1990) and the effect of AR on the production was described, but little is known about the effect of AR on the behaviour of the pigs. As the activity of pigs is related to the heat production, *Diemen et al.* (1995) measured the heat production of the pig treated with *P. multocida* toxin. It seemed to suppress the general well-being of pigs, reducing pigs' activity and food intake. These results indicate the behavioural changes of the pigs suffered by AR but there was no evidence of the behavioural differences. In this study, therefore, the influence of AR connected to behaviour was investigated.

MATERIALS AND METHODS

In the experiment piglets were weaned from their dam at the age of one day and were reared artificially. The computer controlled feeding system provided adequate quantity of milk replacer every hour. Piglets were reared free from the pathogenic agents of AR under controlled animal house conditions. The study contained 14 control and 14 infected piglets, housed separately excluded any contact between the two groups. Piglets in the infected group were pre-treated with 0.5 ml of 0.5% acetic acid in PBS instilled intranasally into each nostril at 6 and 7 days of age, and then were inoculated with 0.5 ml per nostril of toxigenic *P. multocida* suspension (10^5 CFU/ml) at 8 days of age.

The condition of the nasal turbinate bones was examined at the level of the first premolar teeth by computed tomography (CT) just before the infection and after the infection for 12 days (*Magyar et al.*, 2003).

The animals were weighed regularly and on day 8 after infection 9-hour video recordings were made of both groups in parallel by digital cameras. The recordings were analysed using the method of *Molnár et al.* (1998). During the analysis of the video records the appearing frequency data were recorded in every minute. The examined behaviour forms were feeding, drinking, resting, social behaviour, scratches and playing. The behaviour forms were standardized according to *Czakó* (1985).

Feeding behaviour includes the exploration of the food, the recognition, nearing and consuming. The drinking behaviour means simply the water consumption from the drinker. Resting is a neutral stage of the locomotory system which is perceivable from its special position. Resting is frequently interrupted playing but the duration and intensity of these forms is lower compared when they emerge independently.

Social behaviour is a synthetic definition for all the interactions between the individuals of the same group. In our observations, the aggression and the greeting of another animal were listed into this behaviour form. Scratching is behaviour connected to the cleaning and ordering the integument and emerges independently from resting with a high intensity. This form was used only for the scratches itself, in other cases the occasion was identified as social behaviour.

Similarly to the social behaviour, playing is also used as a synthetic definition for different behaviours having an important role during the ontogenesis and the development of the locomotory senses, but has no practical aim.

Playing means generally the manipulation of the food, water and different equipments.

Six behaviour patterns have been observed, in practical terms their presence or absence in a given moment as well as their frequency (i.e. how many of the 14 piglets showed the given behaviour). The data of each behaviour forms was merged and compared to the summarized value of all behaviour forms. Statistical analysis was done by χ^2-probe and percentage rates and the weight means were compared by two-sample independent *t*-test with *SPSS for Windows 10.0* (2001) programme package.

RESULTS AND DISCUSSION

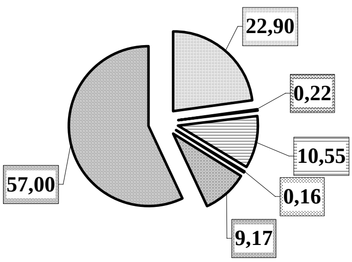
Infection with *P. multocida* after pre-treatment with acetic acid induced the progressive form of AR. In the infected group, the nasal lesions were already severe at 20 days of age as shown by computer tomography. In this short observation period no significant difference was in the mean weights of the groups (3.22±035 and 3.01±0.48 kg; P=0.22). Lower food intake caused by AR might be explained with the reduced activity (*Diemen et al.,* 1995). This assumption was examined by observations. The frequencies of the observed behaviours are shown on *Figure 1*. The highest ratio was set out by resting, which was followed by feeding, social behaviour and playing.

Drinking in both groups was negligible with 0.22 and 0.26% in proportion of the available time. Occurrence of scratching was also minimal (0.16 and 0.11%). Difference between groups was not statistically significant. The control group rested 57% of its time, while the infected group spent significantly more time, 65.7% with resting (chi^2=103, df=1, P<0.001). This difference arose mainly from the 6% decrease in social behaviour (chi^2=181, df=1, P<0.001). In playing, reduction reach 1% (9.17 and 8.32%, N.S.), but frequency of feeding – though only with 1.6%- significantly (chi^2=4.69, df=1, P<0.05) decreased in experimental group (22.9 and 21.3%).

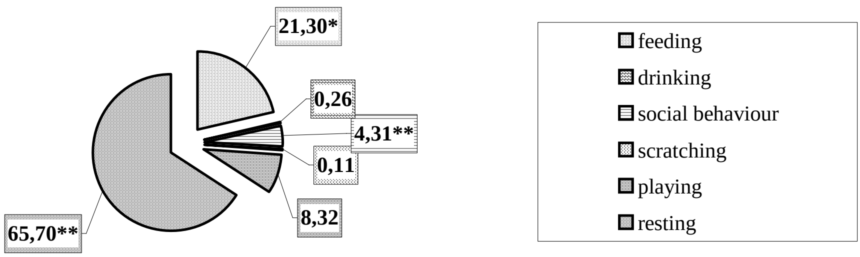
Figure 1

Frequency of the observed behaviour of piglets on day 8 after infection (%)
(* P<0.05; ** P<0.01)

Control group



Infected group



The 8.7% deviation in activity might not be caused by drinking and scratching as their common appearance was almost the same in both groups.

The 1.6% shortening of feeding time mainly was caused by the higher usage of creep feed troughs by control piglets. While in the experimental group only one animal used the trough at a time and for only a short period of time, in the control group the presence of three or four animals at the trough was typical.

The same difference was obvious in the frequency of playing, which is the procedure of exploration behaviour in extensively kept stocks (rummage and massage of the nipples). The use of the nose plays an important role in these forms of behaviour as well, however this was markedly important in social behaviour, where the result of the control was significantly higher with 6.24%. This could be caused by two reasons, which explain the incident in conjunction with each other. The first is the already discussed usage of the nose, which is greatly limited by the pain caused by inflammation in the experimental group, and piglets probably avoid behaviour which is related to the stronger use of the nose. The other reason could be the general spleen caused by the illness.

The biggest difference was experienced in social behaviour. This form of behaviour is a part of comfort behaviour, which is shown by the animals when felt secure and well being. The infected animals were visibly discomforted, woozy, despondent, and supposedly they were not in the mood for chasing and fighting. In hierarchical fights piglets also use their nose.

Beside the quantitative differences there were qualitative deviations in social behaviour. While in the experimental group this meant chewing the tail or ear of each other, in the control group the proportion of hierarchical fights was higher and chasing could be observed, when in many times every animal in the group was running around in the nursing cage. This behaviour was totally absent in the experimental group, and lower activity was general in their social behaviour. On many occasions the animals lay down or stood about while chewing each other.

CONCLUSIONS

On day 8 after *P. multocida* infection we observed that the infected piglets spent less time with eating and social activities than the control piglets while the time they spent with resting increased.

In the literature, only *Drummond et al.* (1981) referred to the reduction of feed intake caused by AR induced with *B. bronchiseptica*. Our examination gives solid evidence that reduced activity coincides with reduced time spent with feeding by the animals. We assume that this is probably caused by the despondency of the ill animals.

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Relationship between the time of separation and the growth performance of artifially reared Awassi lambs

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ABSTRACT

Twenty-one artificially housed Awassi lambs' growth performance (body weights – BW; average daily gain – AVG) was investigated in the first 4 weeks. Animals were divided into three groups (seven lambs in each group) depending on the time of separation. Lambs in the first group were separated immediately after lambing (IW – immediately weaned). Seven lambs were removed 6 hours (6H) and other seven animals were removed 12 hours (12H) from dams after parturition. From the first week, live weight of IW lambs were significantly higher than 12H lambs (P<0.05 at 1 wk age; P<0.001 at 2, 3 and 4 wk age). The same tendency was found evaluating the ADGs. IW lambs had the highest ADG at 1, 2, 3 and 4 wk of age and the differences were significant compared to the values of 12H lambs (P<0.001). Regarding to the differences of body weights and ADGs of IW and 6H groups, no distinctions were found during the whole experiment. On the other hand, 6H lambs had higher body weights than 12H lamb from the 2nd wk (2 wk age: P<0.01; 3 wk age: P<0.001; 4 wk age: P<0.01) and it was the same regarding to ADGs, too (1 wk age: P<0.001; 2 wk age: P<0.01; 3 wk age: P<0.001; 4 wk age: P<0.01). IW lambs showed the highest average ADG (app. 230 g/4wk) and BW (9.98 ± 2.57 kg) at the end of the experiment.
(Keywords: Awassi sheep, artificial lamb rearing, growth performance)

INTRODUCTION

Mutton has traditionally very important role in the food supply of the Mediterranean and Middle-East countries. Awassi is a widespread fat-tailed sheep breed of these regions, with relatively high milk productivity (*Epstein*, 1987). Production of this breed is mainly based on semi-intensive systems with low prolificacy and high milk production (*Emsen et al*., 2004). Whereas, intensive milk producing systems has become widespread in many countries to increase the milk productivity. As it is well-known from literature, Awassi sheep is able to accommodate to the conditions of intensive keeping system (*Pollott* and *Gootwine*, 2001). In addition, artificial lamb rearing is a common keeping method in intensive milk-production systems (*Napolitano et al*., 1995; *Martin*, 1999), which forces lamb separation from dams as soon as it possible. This technology based on early weaning and artificial rearing of lambs on milk replacer and it has an important impact on increasing flock productivity. One of the main factors which have effect on the early development of lambs is the time of weaning. The first 10–12 hours post-partum is a critical, sensitive period during which suckling plays a key role for the establishment of the mother-lamb bond (*Alexander et al*., 1986; *Napolitano et al*., 1995; *Nowak et al*., 1997; *Fisher* and *Matthews*, 2001). It is known that lambs permanently nursed by their dam do not accept being bottle-fed and do not socialise to humans at a young age, despite regular short sessions of human petting (*Boivin et al*., 2001).

As expected from literature, lambs separated early from their mother, readily accepted additional human contact including drinking from a bottle or a bucket of milk (*Markowitz et al.*, 1998; *Boivin et al.*, 2001). Awassi sheep has recently been introduced in Hungary as well (by Bakonszeg Awassi Ltd.), based on an Israeli flock imported in 1989. This breed indicates favourable results in Hungary, primarily in intensive milk production. Whereas, technological difficulties arose, related to the handling of a few lambs. These lambs were not removed from dam immediately after parturition (birth at late-night or early dawn) and were not accept bottle-feeding and artificial teats. Therefore, the aim of this experiment was to investigate the effect of weaning time on early development and growth performance of lambs, considering to the different separation moments during the sensitive period (10–12 h postpartum).

MATERIALS AND METHODS

Twenty-one artificially reared Awassi lambs were divided into three groups with seven animals in each group. IW (immediately weaned) lambs were separated from dams immediately after parturition. These animals were bottle-fed by colostrums three-times a day and were trained to accept the artificial nipple in the first two days postpartum. Other two groups (6H and 12H) were moved from dams 6 and 12 h after parturition and were allowed to suck their mother until that time. After that, 6H and 12H lambs were also trained by the stockpersons to accept the artificial teats. All the lambs were fed with colostrums in the first two days and each group was kept in a 4 m×2 m straw bedded pen. From the 3rd life day, lambs had got also milk powder and its percentage was continuously increased until the 6th life day. From the 14th day of their life, all the lambs had ad libitum access to commercial lamb starter diet and alfalfa hay. Milk replacer was mixed and portioned by ALFA-LAVAL milk equipments which were cleaned and disinfected daily. Used straw of the pens was cleaned and changed; the fence of pens was cleaned and disinfected each morning. Body weights (BW) were measured daily in the first week, than weekly for 4 weeks. Average daily gains (ADG) were calculated from the differences of weights. Data were evaluated by variance analysis (ANOVA) using linear statistical model.

RESULTS AND DISCUSSION

Body weights (BW) of lambs at different ages are shown in *Figure 1*. Significant differences were not found between birth weights of the three groups. At the end of the 1st wk, IW lambs were heavier ($P<0.05$) than 12H lambs, but significantly difference was not found between IW and 6H lambs' body weights at that time.

From the 2nd wk until the end of investigation, very strong significance occurred between live weights of IW×12H and 6H×12H animals (occasionally: $P<0.01$ and $P<0.001$) but no differences were found at IW×6H groups during the whole experiment. From the end of the 1st wk, lambs from 12H group had the lowest live weights until the end of the experiment ($BW_{12H\ at\ 4wk}= 5.35 \pm 0.31$ kg; $BW_{6H\ at\ 4wk}=8.04 \pm 1.73$ kg; $BW_{IW\ at\ 4wk}=9.98 \pm 2.57$ kg). The same tendency evolved at average daily gains (ADGs) which are shown at *Figure 2*. The ADGs of IW group had not varied significantly from 6H group during the experiment. Whereas, differences between IW×12H and 6H×12H groups were very strong, from the beginning to the end of the investigation (occasionally: $P<0.01$ and $P<0.001$). Mean of AVGs of the 4 wk was the lowest at group 12H (app. 60 g/4wk), while the same value was much higher at the other two groups (IW: app. 230 g/4wk; 6H: app. 180 g/4wk).

Figure 1

Body weights of lambs at different ages (IW: immediately weaned; 6H: separated 6 h post-partum; 12H: separated 12 h post-partum). Bars with different letters are significantly different (a-a: NS, non significant; a-b, a-c and b-c: P<0.05)

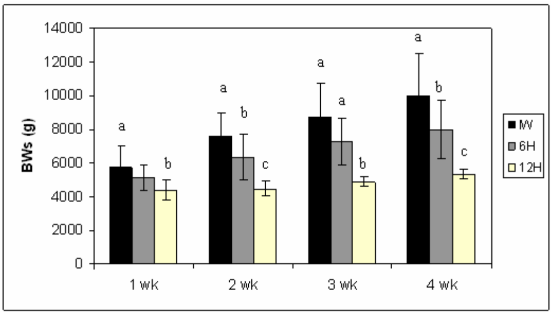
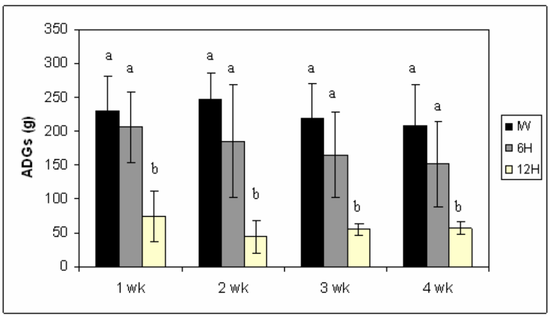


Figure 2

Changing of lambs' average daily gains (ADGs, means and standard deviations) during the investigation (IW: immediately weaned; 6H: separated 6 h post-partum; 12H: separated 12 h post-partum). Bars with different letters are significantly different (a-a: NS, non significant; a-b: P<0.05)



Two of 12H lambs passed away at 3 wk age, because of very strong leakage and low resistance against diseases. These animals were not able to accept bottle-feeding and the artificial nipples. Stockpersons tried to feed them by hypodermic syringe for 5 or 6 days but the amount of milk replacer what they accepted in this way was not enough to survive. As in this study found, it was very difficult to bottle-feed the animals which were able to suck the dam in the first 10–12 hours. Comparing the BWs and ADGs of the investigated groups, we can tell that the presence of dam in the first few hours has had very strong effect on the development of the animals. IW and 6H animals showed significantly higher results both in BWs and ADGs than the 12H lambs. It means that the time of separation influences the parameters of growth performance. The critical period of weaning is between 6th and 12th hour post-partum, as it is also known from previous literature. Additionally to this study, behavioural observations were also done on the artificially reared lambs and the evaluation of data is under process. It would be interesting to identify the effects of keeping method on the behaviour of lambs, with special regard on the meat production parameters.

CONCLUSIONS

Immediate separation after parturition and direct training can help the lambs to accept the artificial nipple and accommodate to the new rearing conditions. As the results show, that was more difficult to feed those lambs which had the chance to suck for a few hours, compared to the immediately separated animals. IW and 6H lambs accepted easier the artificial nipple then 12H lambs, and IW lambs showed the best growth performance in this study. Growth performances were significantly different between the selected groups, and it means that the presence of dam has had very strong effect on the development of the lambs.

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Prediction of carcass composition based on specific carcass cuts in Simmental bulls

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ABSTRACT

Carcass data from 872 Simmental bulls were analysed to estimate the possibilities for prediction of muscle, fat and bone weight as well as percentage in the carcasses from measurement of specific cuts. The right carcass side was first cut into chuck, shoulder, front shank, rib roast, back, loin, tenderloin, brisket, rib, flank, leg and hind shank. Each specific cut was further separated into muscle, fat, bone and tendon. Data were analysed by multiple stepwise regression procedure. As independent variables dissected carcass side weight, weight and the percentage of specific cut and weight and the percentage of different tissues in a specific cut and their quadratic terms were included. The highest coefficient of determination was obtained from leg (for muscle, fat and bone weight 0.9678, 0.839, 0.7972 and for muscle, fat and bone percentage 0.7821, 0.7993, 0.5857). The carcass weight had no effect on average bias, whereas muscle percentage was underestimated in very lean carcasses and overestimated in very fatty one and vice versa for the predicted fat percentage.
(Keywords: beef, carcass, prediction, tissue weight, tissue proportion)

INTRODUCTION

One of the most important factors that define carcass value is carcass composition (*Augustini et al*., 1987). Hence the measurement of muscle, fat and bone content in the carcass is important for meat industry as well as for cattle breeding. The most accurate method for determining the carcass tissue composition is to weight the dissected carcass tissues. However this method is labour intensive and very costly and used only when high accuracy is needed (*Temisan*, 1987). In general, there are two types of prediction equations; it is possible to predict the weight or the proportion of different carcass tissues (muscle, fat and bone). A simple and accurate method for the prediction of carcass composition would bring reduction of labour and costs.

The purpose of this work was to estimate the possibilities for prediction the carcass composition from the composition of specific carcass cuts in Simmental bulls.

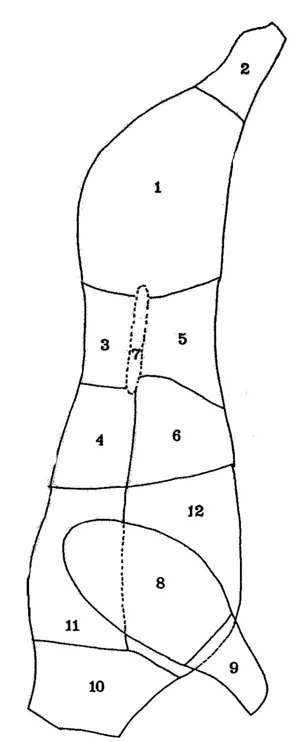
MATERIALS AND METHODS

Data from 872 Simmental bulls from progeny testing station were used in the present study. Bulls were fed with maize silage and concentrates and slaughtered at subjectively defined optimal fatness. After slaughter the right carcass sides were cut into quarters between the 6th and 7th rib and further dissected to the following cuts: chuck, shoulder, front shank, rib roast, back, loin, tenderloin, brisket, rib, flank, leg and hind shank

(*Figure 1*). Subsequently the cuts were dissected to muscle, fat, tendon and bone, and the percentage of tissues was calculated. Means and standard deviation for carcass cuts percentage and carcass cuts tissue composition are shown in *Table 1*. The average carcass side weight was 165±13.26 kg with almost 71% muscle, 12% fat and 16% bone. The rest up to 100% represented tendons. Leg and shoulder represented the highest proportion of the carcass. Flank, brisket and rib had the highest fat content and also the highest standard deviations.

Figure 1

Dissection of right carcass side



Leg(1), Hind shank(2), Loin(3), Back(4), Flank(5), Rib(6), Tenderloin(7), Shoulder(8), Front shank(9), Chuck(10), Rib Roast(11) Brisket(12).

The stepwise regression procedure (*SAS*, 1989) was used to predict the weight and percentage of muscle, fat and bone in the carcasses. Independent variables included in the statistical model, were: carcass side weight, weight and percentage of specific cut, weight and percentage of different tissue in specific cut. Accordingly, for prediction of muscle weight or the percentage in carcass from leg the following independent variables and their quadratic terms were included in the model: carcass side weight, weight of leg, the percentage of leg in the carcass, weight of muscle, fat, bone and tendon in the leg and the percentage of muscle, fat, bone and tendon in the leg. All variables left in the model were significant at the 0.15 level.

Table 1

Means and standard deviations for carcass cuts percentage and their tissue composition in Simmental bulls

Carcass cut	Share of carcass cut, %	Carcass cut composition, %		
		muscle	fat	bone
Leg	28.61 ±1.09	76.16 ±2.08	9.2±1.96	13.12±1.01
Hind shank	3.53 ±0.25	39.87±2.28	5.06±2.37	6.65±2.09
Tender loin	2.32 ±0.21	83.17±4.10	16.83±4.10	-
Loin	3.75 ±0.33	68.33±3.89	6.65±3.00	23.09±3.51
Back	5.62 ±0.73	67.66±3.75	11.14±3.83	20.04±2.81
Rib roast	8.22 ±0.98	75.76±3.53	6.96±2.36	15.49±2.63
Chuck	8.86 ±1.00	78.75±3.32	7.10±2.58	12.57±2.03
Shoulder	14.79 ±0.91	72.41±2.49	11.66±2.67	14.45±1.17
Front shank	2.55 ±0.20	41.63±2.71	3.21±1.67	49.78±2.99
Brisket	9.33 ±0.96	59.95±4.42	23.44±5.15	16.61±2.05
Rib	6.46 ±0.91	65.41±4.37	17.37±5.23	17.23±2.43
Flank	5.98 ±0.79	70.09±6.30	25.62±6.30	-
Carcass side, kg	**165.45±13.26**	**70.73±2.34**	**11.97±2.52**	**15.88±1.05**

Statistical model:

$$\hat{Y}_{ij} = b_0 + b_1 * X_1 + b_2 * X_2 + ... + b_i * X_i + e_{ij}$$

$\hat{Y}_{ij}$ = dependent variable, kg or % of body tissue in the carcass,
b_0 = constant,
$b_1..b_i$ = partial regression coefficients,
$X_1..X_i$ = independent variables,
e_{ij} = estimation error.

RESULTS AND DISCUSSION

The coefficient of determination (r^2) and residual standard deviation (σ_e) for predicting weight of muscle, fat and bone in the carcass are shown in *Table 2*. The r^2 for predicting muscle weight in the carcass were all relatively high, ranking from 0.9678 to 0.867. The r^2 for predicting fat weight were lower than for muscle (between 0.8399 and 0.3517) and for predicting bone weight even lower than for fat (between 0.7972 and 0.4541). The highest r^2 and also the lowest σ_e for the muscle and fat weight in the carcass were predicted from leg and shoulder, whereas the lowest r^2 and also the highest σ_e were predicted from hind and front shank. The highest r^2 and the lowest σ_e for the bone weight in the carcass were predicted from leg and shoulder, whereas the lowest r^2 and the highest σ_e were predicted from tender loin and flank. *Fan et al*. (1992) reported slightly higher r^2 but also higher σ_e for muscle prediction using cold carcass weight, muscle weight in the cut and cut weight (r^2 between 0.932 and 0.978 for hip, loin, flank, rib, chuck, brisket, plate and shank).

The coefficient of determination and residual standard deviation for predicting the percentage of muscle, fat and bone in the carcass (*Table 3*) were much lower than for predicting weight of carcass components. The highest r^2 for percentage of muscle, fat and bone in the carcass were predicted from leg and shoulder whereas the lowest muscle and fat percentage were predicted from front shank and bone percentage from tender loin. Rib and flank represent very interesting cuts, because they can be easily cut from hind quarter and have only minor value. Combined together (data not shown), rib and flank are almost as effective in muscle percentage prediction as leg is, and even better in fat percentage prediction than leg. *Fan et al*. (1992) also reported lower r^2 for prediction of muscle percentage (r^2 0.156 from shank and 0.783 from chuck).

Table 2

Coefficient of determination (r^2) and residual standard deviation (σ_e) of models selected by stepwise procedures for predicting muscle, fat and bone weight in the carcass side

	Muscle, kg		Fat, kg		Bone, kg	
	r^2	σ_e	r^2	σ_e	r^2	σ_e
Leg	0.9678	1.8390	0.8399	1.8979	0.7972	0.9715
Shoulder	0.9508	2.2726	0.7734	2.2604	0.7550	1.0683
Brisket	0.9483	2.3261	0.7780	2.2358	0.6024	1.3572
Rib	0.9438	2.4248	0.7820	2.2132	0.6117	1.3428
Flank	0.9422	2.4580	0.7586	2.3305	0.4902	1.5367
Back	0.9387	2.5353	0.7314	2.4596	0.6568	1.2639
Rib roast	0.9330	2.6540	0.7044	2.5787	0.6128	1.3385
Loin	0.9221	2.8613	0.6356	2.8682	0.5968	1.3681
Tender loin	0.9108	3.0532	0.5325	3.2392	0.4541	1.5920
Chuck	0.9097	3.0561	0.4954	3.3750	0.6104	1.3440
Hind shank	0.8971	3.2836	0.5117	3.3182	0.6462	1.2794
Front shank	0.8671	3.7301	0.3517	3.8189	0.6977	1.1848

Table 3

Coefficient of determination (r^2) and residual standard deviation (σ_e) of models selected by stepwise procedures for predicting muscle, fat and bone percentage in the carcass side

	Muscle, %		Fat, %		Bone, %	
	r^2	σ_e	r^2	σ_e	r^2	σ_e
Leg	0.7821	1.0989	0.7993	1.1321	0.5857	0.2134
Shoulder	0.6684	1.3542	0.7146	1.3502	0.4595	0.2437
Brisket	0.6459	1.4015	0.7132	1.3517	0.0479	0.3225
Rib	0.6161	1.4563	0.7293	1.3157	0.1068	0.3128
Flank	0.6151	1.4581	0.7065	1.3699	0.3360	0.2705
Back	0.5746	1.5337	0.6582	1.4783	0.1547	0.3046
Rib roast	0.5396	1.5947	0.6281	1.5404	0.0756	0.3184
Loin	0.4715	1.7125	0.5473	1.7034	0.0752	0.3185
Tender loin	0.3956	1.8261	0.4162	1.9289	0.0322	0.3256
Chuck	0.3918	1.8450	0.3617	2.0204	0.1165	0.3116
Hind shank	0.3017	1.9685	0.3869	1.9812	0.2725	0.2826
Front shank	0.0971	2.2345	0.1884	2.2768	0.1697	0.3025

To test the accuracy of prediction, we plotted residuals together with carcass side weight and carcass fat percentage. As an example we chose the prediction of percentage of muscle, fat and bone in the carcass from leg.

Figure 2

The effect of carcass side weight residuals of estimated meat percentage from leg

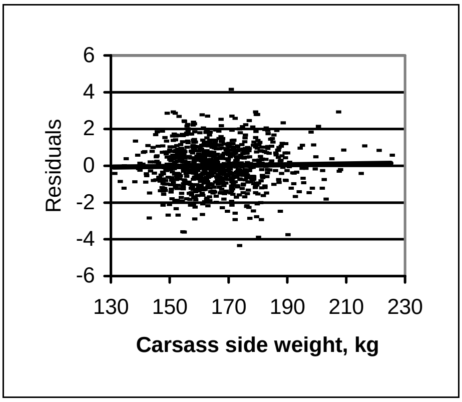
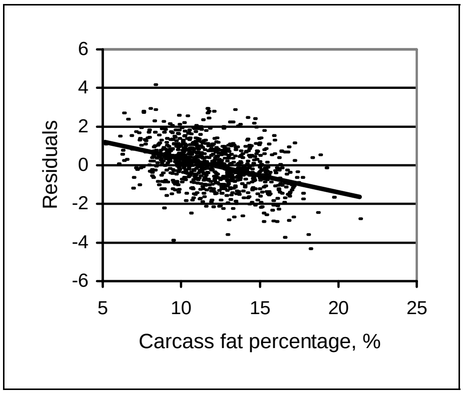


Figure 3

The effect of carcass fat on residuals of estimated meat percentage from leg



Figures 2, *4* and *6* evidently show that carcass side had no effect on bias. In contrast to carcass side weight, carcass fat percentage exhibited an effect on bias. The muscle percentage (*Figure 3*) was underestimated in carcasses with low percentage of fat and overestimated in carcasses with high percentage of fat. Just the opposite was true for the predicted fat percentage (*Figure 5*). It was overestimated in carcasses with low percentage of fat and underestimated in carcasses with high percentage of fat. The accuracy of bone prediction was not affected by fat percentage in the carcass (*Figure 7*).

Figure 4

The effect of carcass side weight on residuals of estimated fat percentage from leg

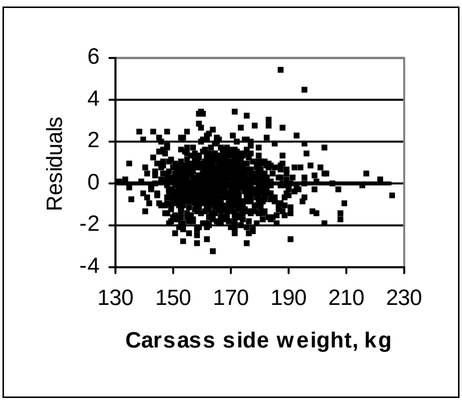


Figure 5

The effect of carcass fat on residuals of estimated fat percentage from leg

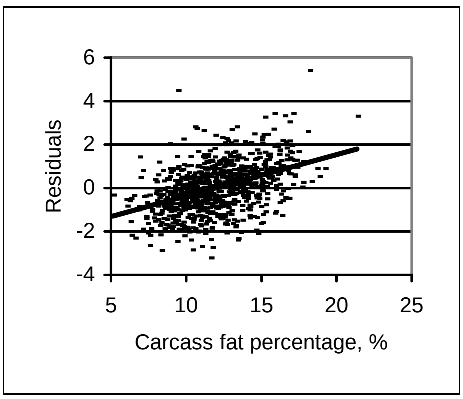


Figure 6

The effect of carcass side weight on residuals of estimated bone percentage from leg

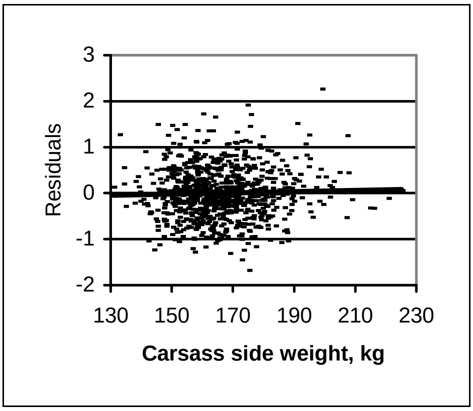
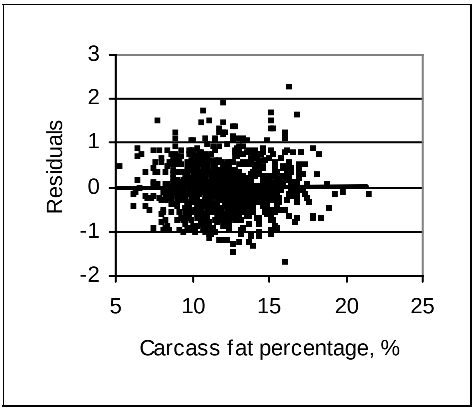


Figure 7

The effect of carcass fat on residuals of estimated bone percentage from leg



CONCLUSIONS

The obtained results lead to the conclusion that the coefficient of determination for muscle, fat and bone weight prediction from carcass side weight and different carcass cuts composition is higher than the prediction of muscle, fat and bone percentage. The highest r^2 and the lowest σ_e were obtained from leg as well as for weight and for the percentage of different carcass tissues. The carcass side weight had no effect on bias of estimated carcass components, whereas the muscle percentage was underestimated in very lean carcasses and overestimated in very fatty ones. Fat percentage was overestimated in lean carcasses and underestimated in fatty carcasses. The accuracy of bone prediction was not affected by fat percentage in the carcass.

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Effect of divergent selection for the volume of thigh muscles based on computerised tomography on the carcass traits of rabbits (preliminary results)

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ABSTRACT

The aim of the study was to examine the efficiency of the selection based on CT measured thigh muscles with divergent selection method. Does of the first generation selected for high or low muscle volume were inseminated with the semen of similarly selected bucks at the same time. At 12 weeks of age 25–25 rabbits of similar body weight were slaughtered and dissected from both progeny groups (L: progenies of the rabbits selected for low volume of thigh muscles; H: progenies of the rabbits selected for high volume of thigh muscles). The gastrointestinal tract weight and its proportion within body weight were 38 g (P<0.05) and 1.2% (P<0.001) lower in H rabbits compared to L animals. Thus, the dressing out percentage in H rabbits was 2% higher (P<0.001). The proportion of the hind part within reference carcass was 1.2% (P<0.001) higher, while that of the fore part was 0.7% (P<0.05) lower in group H. The weight of hindleg meat in H rabbits was 33 g (P<0.001), while its ratio to reference carcass was 1.2% (P<0.001) higher than in L animals. Data of the experiment confirmed the efficiency of CT based selection for thigh muscles. Increasing the volume of hindleg muscles, the dressing out percentage improved as well. However, beside carcass traits, it would be interesting to study the productive traits of the progenies of the first and second generations selected divergently as well.
(Keywords: rabbit, computerised tomography, thigh muscles, two-way selection, carcass traits)

INTRODUCTION

At the University of Kaposvár the Pannon White rabbits are selected for carcass traits with the help of computerised tomography (CT). Previously, the selection was based on the cross-sectional area of the *m. Longissimus dorsi* (*Szendrő et al.*, 2005). The divergent selection (*Szendrő et al.*, 2005) or data analysis by BLUP and REML methods (*Nagy et al.*, 2006) proved the significant increase in carcass traits due to the selection. Comparing the progenies of Pannon White bucks to that of terminal bucks of different hybrids, higher dressing out percentage, higher amount of *m. Longissimus dorsi* and more muscles on hind legs were achieved in the progenies of Pannon White bucks as a result of CT based selection (*Metzger et al.*, 2006). As the weight of the hindleg meat is 2.3–2.5 times larger than that of the *m. Longissimus dorsi*, the base of the selection was changed in 2004; and since then the volume of thigh muscles has been estimated based on 11–12 CT scans. Divergent selection is one of the commonly used methods for examining the efficiency of the selection (*Baselga* and *Garcia*, 2002; *Pla*, 2004). Previously, the selection based on the *m. Longissimus dorsi* was also proven by divergent selection (*Szendrő et al.*, 1996).

The aim of our study was to examine the efficiency of the selection based on CT measured thigh muscles with divergent selection method.

MATERIALS AND METHODS

Animals

Experiment was carried out at the University of Kaposvár on Pannon White rabbits. One part of the breed was divergently selected for high or low volume of thigh muscles measured by computerized tomography (CT). The animals were weighed at 5 and 10 weeks of age, to calculate the average daily weight gain. Only rabbits showing higher body weight gain than the average (45.0 g for female and 47.6 g for male rabbits) were selected for the CT measurements. The CT scans were taken every 10 mm between *tuber sacrale* and *patella*. On the CT scans the muscle area was measured and the muscle weight was calculated. The estimated thigh weight of rabbits selected for high or low muscle volume was 353 and 309 g, respectively.

Divergently selected does of the first generation were inseminated with the semen of similarly selected bucks at the same time. Progenies (born at the same day) were divided into two groups:

- L: progenies of the rabbits selected for low volume of thigh muscles,
- H: progenies of the rabbits selected for high volume of thigh muscles.

Rabbits were weaned at 35 days of age. After weaning, they were reared in fattening cages made of wire mesh until slaughtering (2–3 rabbits per cage). The rabbit house had 15–16 °C average temperature, a 16L:8D lighting cycle and overpressure ventilation. Animals were fed a commercial diet (10.6MJ/kg DE, 16.0% crude protein, 3.0% ether extract, 16.0% crude fibre) *ad libitum*. Drinking water was available *ad libitum* from nipple drinkers.

Slaughtering and dissection procedure

At 12 weeks of age 25–25 rabbits of similar body weight were slaughtered and dissected according to the method of *Blasco* and *Ouhayoun* (1996). Rabbits were weighed immediately before slaughter, then killed by bleeding after electric stunning. Carcasses were chilled at 4 °C for 24 hours, then the chilled carcasses (together with head, heart, lungs, liver, kidneys, periscapular and perirenal fat) were weighed. The heart, lungs, liver and kidneys, and then the periscapular and perirenal fat were removed then weighed. The head was separated from the carcass and it was cut between 7th and 8th thoracic vertebrae and between 6th and 7th lumbar vertebrae, and thus the fore, intermediate and hind parts were obtained. Subsequently the hindlegs were weighed then deboned, and the meat on the hindlegs (HL) was weighed. The *m. Longissimus dorsi* (MLD) was removed from the intermediate part. The ratio of organs and carcass parts to body weight and to reference carcass weight (fore- intermediate and hind parts with fat depots) was calculated.

Statistical analysis

Experimental data were analysed using Independent Samples t-test of SPSS 10.0 program package (*SPSS* for Windows, 1999).

RESULTS AND DISCUSSION

The gastrointestinal tract weight (*Table 1*) and its proportion within body weight (*Table 2*) were 38 g (P<0.05) and 1.2% (P<0.001) lower in H rabbits, resp. Since this is the main loss during the slaughtering process, the dressing out percentage of group H was 2% higher (P<0.001) compared to L animals (*Table 2*).

Table 1

Effect of divergent selection based on CT measured thigh muscles on the weight of organs and carcass parts in the progenies of the first generation

Traits	Selection		Prob.
	L	H	
	mean ± SD	mean ± SD	
No. of rabbits	25	25	-
Body weight, g	3043 ± 140	3040 ± 162	0.941
Skin, g	444 ± 25.2	442 ± 40.0	0.814
Gastrointestinal tract, g	**561** ± 49.3	**523** ± 41.0	**0.005**
Chilled carcass, g	**1764** ± 93.9	**1826** ± 118	**0.046**
Liver, g	**95.6** ± 14.3	**84.9** ± 13.7	**0.010**
Kidneys, g	**19.9** ± 2.98	**17.7** ± 2.32	**0.005**
Heart and lung, g	24.1 ± 4.61	22.8 ± 3.15	0.230
Perirenal fat, g	29.9 ± 13.9	27.6 ± 11.3	0.529
Scapular fat, g	10.4 ± 4.83	8.96 ± 4.19	0.266
Head, g	**138** ± 7.69	**142** ± 7.84	**0.040**
Fore part, g	439 ± 30.6	449 ± 34.9	0.292
Intermediate part, g	*475* ± 33.6	*495* ± 39.7	*0.063*
Hind part, g	**538** ± 28.3	**579** ± 36.1	**0.000**
m. Longissimus dorsi, g	*205* ± 17.3	*216* ± 20.5	*0.055*
Hindleg meat, g	**405** ± 25.7	**438** ± 31.7	**0.000**

L: progenies of the rabbits selected for low volume of thigh muscles.
H: progenies of the rabbits selected for high volume of thigh muscles.

From the edible organs, the weight of liver and kidneys was 10.7 (P<0.01) and 2.2 (P<0.01) lower in H animals, resp. (*Table 1*). Neither the weight nor the ratio of fat depots changed due to the selection (*Table 1* and *Table 2*).

Examining the weight and the ratio of carcass parts, it can be established that the hind part of H animals improved at the expense of the fore part which is the most bony part within the carcass. The proportion of the hind part within reference carcass was 1.2% (P<0.001) higher, while that of the fore part was 0.7% (P<0.05) lower in group H (*Table 2*). The proportion of the intermediate part within reference carcass did not differ (*Table 2*). The weight of the *m. Longissimus dorsi* was 11g higher (P=0.055) in H rabbits (*Table 1*), however, its ratio to reference carcass did not differ significantly (*Table 2*).

Since the selection was based on the volume of thigh muscles, thus, the development of hindleg muscles in the progenies of divergently selected groups had main importance. The results proved our expectations, since the weight of hindleg meat in H rabbits was 33 g (P<0.001) (*Table 1*), while its ratio to reference carcass was 1.2% (P<0.001) (*Table 2*) higher than in L animals.

Table 2

Effect of divergent selection based on CT measured thigh muscles on the proportion of organs and carcass parts in the progenies of the first generation

Traits	Selection		Prob.
	L	H	
	mean ± SD	mean ± SD	
Ratio to body weight, %			
Skin	14.6 ± 0.63	14.5 ± 0.96	0.763
Gastrointestinal tract	**18.4** ± 1.19	**17.2** ± 1.15	**0.001**
Chilled carcass (Dressing out percentage)	**58.0** ± 1.42	**60.0** ± 1.47	**0.000**
Ratio to reference carcass weight, %			
Perirenal fat	1.99 ± 0.90	1.70 ± 0.67	0.212
Scapular fat	0.69 ± 0.32	0.56 ± 0.26	0.138
Fore part	**29.4** ± 1.24	**28.7** ± 1.16	**0.049**
Intermediate part	31.8 ± 1.13	31.8 ± 1.22	0.945
Hind part	**36.1** ± 1.00	**37.2** ± 0.83	**0.000**
m. Longissimus dorsi	13.8 ± 1.01	13.9 ± 1.00	0.690
Hindleg meat	**27.1** ± 1.02	**28.2** ± 0.64	**0.000**

L: progenies of the rabbits selected for low volume of thigh muscles.
H: progenies of the rabbits selected for high volume of thigh muscles.

Previously, the selection of Pannon White rabbits based on the cross-sectional area of *m. Longissimus dorsi* (*Szendrő et al.*, 2005). The cross-sectional area determined *in vivo* by CT between the 2nd and 3rd and between the 4th and 5th lumbar vertebrae (L-value) was positively correlated to the dressing out percentage and to the weight of the meat on loin and on hindlegs (r=0.65-0.67) (*Szendrő et al.*, 1992). Comparing the carcass traits of the progenies of bucks selected divergently on L-value, *Romvári* (1996) established that the dressing out percentage improved (in the progenies of '-' sel, '+' sel and '++' sel bucks: 62.3, 63.1 and 64.1%, resp.), the weight of the intermediate part increased (in the progenies of '-' sel, '+' sel and '++' sel bucks: 430, 433 and 452g, resp.), while the gastrointestinal tract weight decreased (in the progenies of '-' sel, '+' sel and '++' sel bucks: 379, 364 and 356g, resp.) due to the response to selection. The efficiency of the selection based on L-value is proven by the yearly 1.65% genetic trend (*Nagy et al.,* 2006).

The correlation between CT measured and weighed (during slaughter) hindleg meat was close (r=0.71) (*Szendrő et al.*, 2005). Our results proved the efficiency of selection, since the weight of hindleg meat was significantly higher in H animals compared to L rabbits. Due to the increased weight and ratio of hindleg meat in H rabbits, the proportion of hind part within carcass as well as dressing out percentage also increased in this group. Thus, the most meaty part increased due to the selection, which is advantageous.

Our results prove that the efficiency of CT based selection for thigh muscles is similar to that of for L-value (*Szendrő et al.*, 1996; *Szendrő et al.*, 2005; *Nagy et al.*, 2006). Since the weight of hindleg meat is 2.3–2.5 times larger than that of *m. Longissimus dorsi*, thus, the meat production of growing rabbits could improve to a greater extent in case of similar genetic trend was found in L-value.

CONCLUSIONS

Data of the experiment confirmed the efficiency of CT based selection for thigh muscles. Increasing the volume of hindleg muscles, the dressing out percentage improved as well. The proportion of hind part within carcass increased at the expense of the fore (the most bony) part in the progenies of the rabbits selected for high volume of thigh muscles, which is advantageous. Since both the weight and the ratio of gastrointestinal tract were significantly lower in H rabbits, it would be interesting to study not only the carcass traits but also the productive traits in the progenies of the first and second generations selected divergently.

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Growth and feed conversion of intensively reared Volga perch (*Stizostedion volgensis)*

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ABSTRACT

Intensive rearing technology of pikeperch has been developed significantly in Central Europe in the last few years. The variety of species producing excellent quality meat can be widened by the Volga perch, a fish that shows slower growth than pikeperch but much higher tolerance to suboptimal environmental conditions. The experiment to test the growing capacity of pond pre-reared Volga perch was carried out at the fish laboratory University of Kaposvár. Fish were held in 130 L aquaria, setting three stocking densities (1.25; 1.66; 2.08 g/l). The experiment lasted for 6 weeks. Our results suggest that rearing of Volga pikeperch alevins based on commercial trout pellet feeding is viable. Stocking density variations applied in our experiment caused no significant differences, neither in feed consumption (18.19±2.79 g; 19.67±1.41 g and 18.92±1.70 g) nor in feed conversion rate (0.86±0.08; 0.85±0.10 and 0.93±0.03 g/g). The effect of the three stocking densities on total weight gain (21.06±1.80 g; 23.35±2.80 g and 20.28±1.29 g, respectively) was not significant (P=0.065). Growth rate, expressed as S.G.R., was found to be 1.62±0.07; 1.72±0.14 and 1.54±0.06%/day, respectively. Difference between the middle and the highest stocking density was significant at P=0.03.
(Keywords: Volga perch, feeding, stocking density, intensive rearing)

INTRODUCTION

Volga perch is a very little known species of the European fauna. Hungarian research work was focused on the Balaton lake population. It was found that in spite of the relatively high fecundity its occurrence ratio is below 1% (*Specziár* and *Bíró*, 2002). Invertebrate fauna is the main feeding source even for the older age groups. Volga perch reaches sexual maturity at the age of 3–4 years in Lake Balaton when generally measures 200–250 mm (*Szipola*, 1994). According the finding of *Specziár* (2002) the 30–100 mm long first-summer-old fish feed mainly on planctonic Cructaceans and bentonic Chironomids. This feeding spectrum suggested that the weaning of fry from zooplankton to artificial feed would be easier than it was in case of pikeperch. The conversion for pelleted feed was found to be successful even with larger (60–100 mm long) fish in an earlier study (*Müller et al*., 2003).

The aim of our experiment was to adapt the rearing methods developed for pikeperch to Volga perch fry and to test the effect of stocking density first.

MATERIALS AND METHODS

The experiment was carried out at the fish laboratory of University of Kaposvár. The experimental stock, already weaned to pelleted feed, was received from the Georgikon Faculty of the University of Veszprém.

Fish were stocked in 130 l aquaria functioning in recirculation system with individual aeration where the water temperature was 22±0.5 °C. Three levels of stocking density were set up with means of 1.25, 1.66 and 2.08 g.l^{-1} in four randomly assigned replications each. The whole experimental stock was weighed and measured individually at the beginning and at the end of the experiment that was continued for six weeks. Data of starting weight and standard body length are summarized in *Table 1*. Commercial trout feed was offered once a day. The composition of the feed was as follows: crude protein: 43.9%, crude fat: 17.4%, crude fibre: 1.3%.

Growth rate of fish was expressed as specific growth rate (S.G.R.) and as daily weight gain. Feed consumption was recorded daily for every aquarium. Condition factor was also calculated at the start (K_s) and at the end (K_e) of the experiment.

Statistical analyses were carried out by SPSS for Windows 8.0 package. Treatment effects were analysed by one-way ANOVA and treatment means were compared by Tukey's test.

RESULTS AND DISCUSSION

The most important production traits achieved in the experiment are shown in *Table 1*. Different stocking density did not cause significant differences in feed consumption (*Figure 1*). It is important to remark that Volga perch, in contrast with pikeperch, is inclined to pick up feed pellets from the bottom of the aquarium.

Table 1

Production traits of the experimental stock (mean ± S.D.)

Trait	Stocking density (g.l^{-1})		
	1.25	1.66	2.08
Initial weight (g)	21.5±1.1	21.9±0.7	22.2±1.3
Initial s. length (mm)	119.4±7.3	118.7±4,7	123.5±6,6
Daily weight gain (g.day^{-1})	0.50±0.04	0.55±0.06	0.48±0.03
Growth in length (mm.day^{1})	0.70±0.19	0.77±0.05	0.63±0.11
Starting condition factor(K_s)	1.28±0.22	1.31±0.1	1.18±0.12
Ending condition factor (K_e)	1.28±0.05	1.30±0.11	1.25±0.02

Mean values of F.C.R. were found to be 0.86; 0.85 and 0.93 g.g^{-1} that shows excellent conversion of the trout feed. Differences were not significant.

Daily weight gain showed the lowest value at the highest density but the differences were also not significant (P=0.065). However the difference of S.G.R. between the medium and high density was proved to be significant (*Figure 2*). Condition factor of fish practically did not change during the experiment and also was not changed by the treatments.

Figure 1

Feed consumption at the different stocking densities

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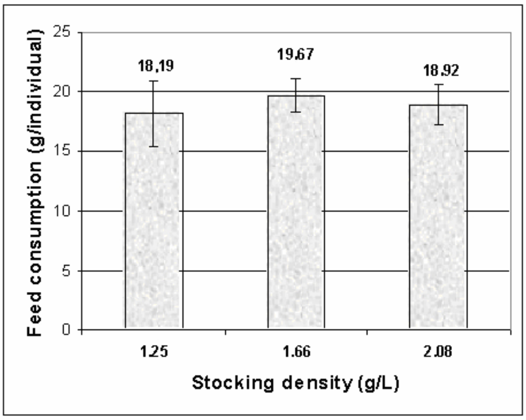
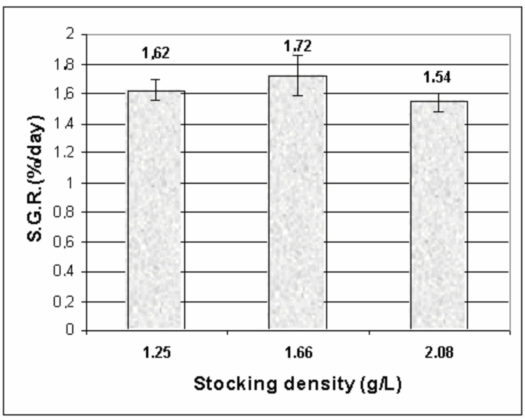


There was no loss of fish during the six weeks of experiment. It is a remarkable difference from pikeperch, however, that Volga perch proved to be more sensitive to the handling stress during measurements. Clear symptoms of shock (with spasmodic bending of the backbone) were observed many times but these fish recuperated within a period of one to two hours when replaced to their aquarium and showed no apparent lasting damage.

Figure 2

Growth rate of fish at different stocking densities



CONCLUSIONS

Numerous studies can be found dealing with the weaning of pikeperch larvae to collected zooplankton and artificial feed. Several types of formulated starter feed were also tested but generally very slow growth and high mortality was observed (*Ruuhijärvi et al*., 1991) or, although larvae consumed the offered feed, they were not able to digest it (*Schlumberger* and *Proteau*, 1991). It can be concluded that the intensive rearing of Volga perch larvae, similarly to the pikeperch, seems to be unviable. Using the simple and cheap method of pond rearing of fry the desired volume of pre-reared fry of about 30 mm body length can easily be produced. This size can already be weaned to pelleted feed with acceptable losses in intensive systems. This transition period was found to be at least two weeks for the pikeperch fry (*Molnár et al*., 2002 a,b). Weaning of Volga perch fry from zooplankton to pellet demands only from 8 to 10 days. Survival of pellet fed pikeperch changes between 44 to 49% in which cannibalism plays a significant role while "natural" causes of losses have a ratio of 8 to 14% (*Molnár et al*., 2004). No cannibalism was observed in this experiment showing that Volga perch has quite different feeding behaviour than pikeperch.

According to our previous results pikeperch alevins fed on trout pellet showed excellent growth rate (S.G.R.=6.5−7%.day^{-1}). Individual feed consumption on the 10th week of life was between 0.17 to 0.20 g that is 16 to 20% of fish biomass. Mean F.C.R. was about 0.8 g.g^{-1}. No data of this kind were found about Volga perch in the literature. *Speczíár* and *Bíró* (2002) estimated the growth rate of +1 age group to be about 2%. day^{-1} in Balaton lake and although this is not really comparable to our present results it is at least interesting that we measured similar values in our above described experiment. Investigating the effect of stocking density on weight gain, feed consumption and F.C.R. on pikeperch no significant differences were found below 2.08 g.l^{-1} stocking density (*Molnár et al*., 2004). Similar results were achieved with Volga perch in the present study.

The idea of producing the hybrid of pikeperch and Volga perch has also emerged (*Müller et al*., 2003). The fish in which the fast growth of pikeperch and the higher resistance of Volga perch would be alloyed should really be ideal for intensive culture. However a lot of further investigations are needed to determine favourable keeping conditions and species specific feeds for the intensive rearing of both of these valuable Percids. To test the fishpond culture of Volga perch also needs to be considered.

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Some slaughter characteristics of the mallard (*Anas p. platyrhynchos*, L. 1758)

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ABSTRACT

Three subspecies of the mallard (Anas p. platyrhynchos, L. 1758) are typical nesting birds in the Holarctic. The mallard is our most important warefowl and will probably keep this position in the future. Because of the very few literature sources it seemed to be reasonable to examine the so called important poultry-industrial characteristics of the mallard in both sexes, like the carcass weight, valuable meat (carcass) parts, slaughter losses etc Altogether 24 mallards shot were examined. After the wet placking the carcasses were opened, eviscerated and dissection according to the method used in poultry processing (Jensen, 1983). The body weight (shot) and weight of brest and thighs with skin as well as the weight of brest fillet and thigh fillet were weighed. The average values of the carcass weight for the two sexes were ♂1228±160 g; ♀1058±98 g. The weight data of the breast with skin were 256±33 g in drakes and 239±36 g in ducks($p<0.05$). The breast fillet weight were 167±28 g and 153±24 g respectively. The weight data of the thighs with skin were 138±14 g in drakes and 119±12 g in ducks. The weight data of the thigh fillet were ♂80±12 g and ♀65±6 g respectively ($p<0.05$).
(Keywords: Anas platyrhynchos, mallard, slaughter parameters, breast filet, thigh filet)

INTRODUCTION

Three subspecies of the mallard are typical nesting birds in the Holarctic. The basicform, *Anas p. platyrhynchos*, L. 1758 hatch in Europe, Asia and North-America. It is widely dispersed on our continent occurring everywhere in the advantageous biotopes. The European stock is more than 8 Million specimens *Rose-Scott*, cit. *Faragó* (2002) what was stated to be a stable (constant) population by *Tucker* and *Heath*, cit. *Faragó* (2002), however *Cramp* (1984) and *Aubrecht* and *Holzer* (2000) give account of a 4–5 Million stock size for the Western Palearctic. In Hungary mallard is common in every watery biotope and overdispersed during migration and wintering if the wether conditions are optimal. The Hungarian breeding stock was estimated to be 100–150 thousand pairs by *Magyar et al*., cit. *Faragó* (2002).

The mallard is our most important warefowl and will probably keep this position in the future. The annual bag is varying between 60–90 thousand specimens (*Csányi* cit. *Faragó*, 2002). Due to the bird's abundance beside its importance in the game management, it is an important pray for many predators according to *Ruiz-Olmo* and *Marsol* (2002).

In regard of the above mentioned circumstances and in the knowledge of the very few literature sources it seemed to be reasonable to examine the so called important poultry-industrial characteristics of the mallard in both sexes, like the valuable meat (carcass) parts, slaughter losses etc. as well as the species role in the predators' food-menu. The investigation of this later subject is undergoing but not viewed in this article.

MATERIALS AND METHODS

Altogether 24 mallards shot in several (dawn) on the water dum of the Tapsony Hunting Club (Somogy County, Hungary) in October 2005 were examined. The total bag consisted 7 drake and 17 duck.

The birds (carcasses) were processed in the special room for poultry experimental slaughter of the Department of Poultry- and Pet Breeding of the University of Kaposvár. After the wet placking the carcasses were opened, eviscerated and dissection according to the method used in poultry processing (*Jensen*, 1983). The body weight (shot) and weight of brest and thighs with skin as well as the weight of brest fillet and thigh fillet were weighed to the nearest gramm on a digital scales.

The carcass weight and the slaughter characteristics data were analysed by independent samples T-test using the SPSS 10.0 For Windows (1999) statistical program package.

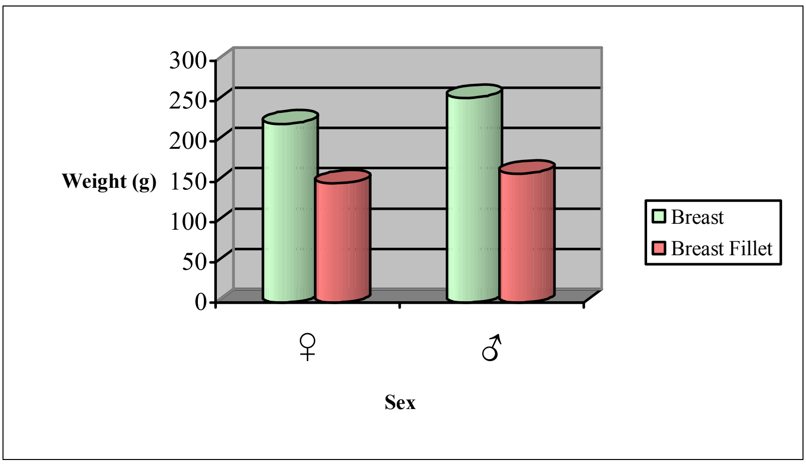
RESULTS AND DISCUSSION

The carcass weight

The average values of the carcass weight for the two sexes – ♂1228±106 g; ♀1058±98 g – falled between the ranges of the adult liveweight values, found in the literature (*Cramp*, 1984). The liveweight data are comparable with the shot (dead) weight data by our opinion, because there is no loss practically in the weight of the bird body killed by small shot (pellett). The 15% difference experienced between the averages of the two sexes is also well suits with the literature data.

Figure 1

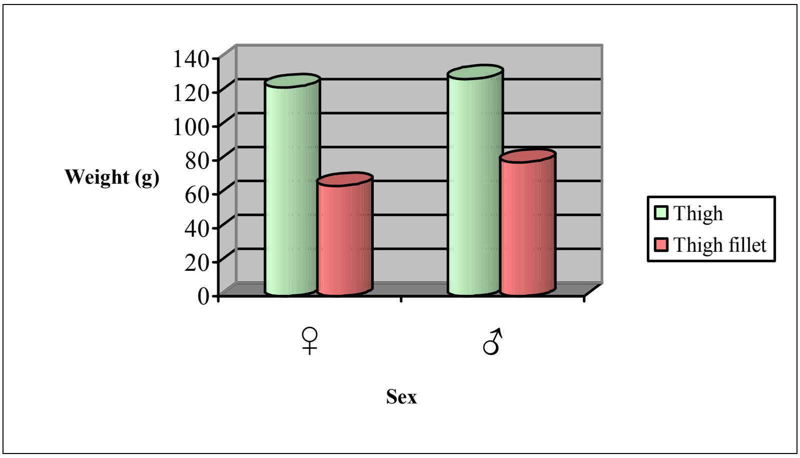
Development of the weight of breast (with skin and bone) and breast fillet in both sexes in mallard



The weight data of the breast in skin were 256±33g in drakes and 239±36g in ducks. The difference between the sexes cannot prove significantly. These data are among the ones given by *Golze* and *Damme* (2001): 309 g and 272 g; and those of *Golze* and *Schröder* (2001): 207 g and 201 g (*Figure 1*). The breast fillet weight were 167±28g in drakes and 153±24g in ducks respectively also without significant difference.

Figure 2

Development of the weight of thighs (with skin and bone) and thighs fillet in both sexes in mallard



The weight data of the thighs with skin were 138±14 g in drakes and 119±12 g in ducks (*Figure 2*). The difference between the sexes was not significant statistically. These data are below the ones (those) found in the literature: 153:131 g (*Golze* and *Damme*, 2001); and 145:134 g *Golze* and *Schröder* (2001). However these records originate from a stock of intensively reared 8 week old mallards.

The weight data of the thigh fillet were 80±12 g in drakes and 65±6 g in ducks respectively. The difference between the two sexes cannot prove significantly.

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Step characteristics analysis of Lipizzaners in Croatia

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ABSTRACT

Due to the increased trend of using Lipizzaner in driving sport in Croatia, the goal of the research was to improve the measuring of characteristics which determine Lipizzaner physical performance using digital video camera. The length and speed of walk and trot on hand were analyzed for Lipizzaner mares and stallions owned by Lipizzaner Stud of Đakovo and private breeders in Croatia. In three repetitions we measured the length of walk and trot step of 71 head of Lipizzaner breed as well as withers height, chest girth and cannon bone circumference. By analyzing step characteristics we obtained the shortest time needed for one step per second (0.97 s/s for stallions and 0.92 s/s for mares) in relation to literature about Lipizzaner breed, and the top speed in meters per second (1.65 m/s for stallions and 1.,66 m/s for mares). The length of a walk duration for Lipizzaner mares was 0.92 step per second and for stallions it was 0.97 step per second. The length of a trot step duration for Lipizzaner mares was 1.38 step per second and for stallions it was 1.46 step per second. The length of step on 50 meters for Lipizzaner mares was 1.78 meters and for stallions it was 1.69 meters. The length of trot step on 50 meters for Lipizzaner mares was 2.52 meters and for stallions it was 2.51 meters. Walk speed at kilometer per hour on 50 meters was 5.90 km/h for mares and stallions together. Average trot speed was 12.57 km/h for mares and 13.23 km/h for stallions.
(Keywords: Lipizzaner horses, step characteristics, driving sport)

INTRODUCTION

The Lipizzaner Stud of Đakovo is the only State Horse Herd for Lipizzaner in the Republic of Croatia that participates in world breeding with 13%. In Slavonija and Baranja there is a centuries' long tradition of Lipizzaner horse breeding by private breeders and there is also a recently increased trend of using Lipizzaner in driving sport. Thus, the goal of the research was to improve the measuring of characteristics which determine Lipizzaner physical performance by using digital video camera. Service value of a horse depends on its walk. The walk is conditioned by built and horse training. A proper, steady, rich, balanced and flexible walk is desired. A horse with a good walk should have harmonious built, strong muscles, appropriate body mass, good training and adequate care. Rectangular body shape causes longer and richer walk, square body shape causes shorter and less rich walk. Short walk is often the consequence of steep shoulder blade and short humerus. Nice and gracious walk is the consequence of high leg action. The goal of the research was to analyze the length of walk and trot step and the walk and trot speed on 20 and 50 meters. According to *Barrey* (2001) locomotive apparatus is a complex consisting of systems that include muscles, bones and joints that are controlled by central nerve system and the result is very good movement. Biomechanically moving includes movements of the entire body and parts of the limbs in rhythmical and mechanical connections, which defines different walk. It is known that heritability of conformation of body and legs is high. *Arnason* (1984), *Preisinger* and *associates* (1991), *Saastamoinen* and *Barrey* (2000) established that the subjective evaluation of conformation is influenced by non genetic factors such as judges' experience, sex and age of head, physical condition, evaluation period etc. The subjectively evaluated characteristics are conformation of body and legs, movements and orthopedic characteristics as opposed to objectively evaluated characteristics obtained by using different photography and video techniques. According to *Clayton* and *Schamhardt* (2001) photometric methods have improved by using digital video camera and analyzing individual pictures and they are based on measuring angels and length of bones.

Barry and *associates* (1993) took linear measurement and the choice of moment of the last trot and hurdle jump from video sequences. *Giovagnoli* and *associates* (2001) made the shooting of treadmill exercise using surface videoelectromiographical shooting. Jumping parameters, according to *Lewczuk* (1997), were measured using non automatic basic programs for video analysis. *Holmström* (1990) proves that newer techniques of digital photography and shooting horse movement open new possibilities for objective assessment of body measures, for relation between movement centers and axes angles of locomotive system and for making the appropriate documentation. Digital video recording is especially suitable for objective movement assessment either for walk, trot or gallop. Team of judges estimate locomotion characteristics in young horses such as step length, amplitude, walk flexibility, ability for training and hurdles (*Saastamoinen* and *Barrey* 2000).

MATERIALS AND METHODS

For testing the length and speed of walk and trot step 102 Lipizzaner thoroughbred head over three years of age were recorded. Because of walk "purity" 71 head were analyzed. A professional cameraman was recording with digital Sony 200 camera (DVKEM) on all locations (Babina Greda, Sikirevci, Svilaj, the State Stud of Đakovo and stud fare). The recording was made from the left and from 50 meters distance in order to have a clear picture of the whole horse and its movement at all times. The manner of recording on all five locations was identical considering the summer weather conditions, level, grass, dry surface, professional cameraman and the crew. In three repetitions we measured the length of walk and trot step. On the 50 meters run 12 cones were placed from the start (0 meters) and then on every 10 meters to 50 meters on finish. The distance between cones was 8 meters, so that horses would not be frightened by unknown shape of the cones and their bright red color. Such cones are usually placed as "gates" in pair driving.

On start, 20 meters length and finish at 50 meters were placed people who marked the head passing through start and finish, as well as people who measured time by stopwatch.

Withers height, chest girth and cannon bone circumference were band measured in the month when the step testing was conducted. Differences in arithmetic mean for step characteristics between different sexes were tested by t-test (PROC TTEST). Since the variance equivalence presumption was wrong in two walk speed on 20 or 50 meters tests, indicated by F values, latitude level was corrected according to *Satterthwait* (1946).

Following parameters were taken into account for analyzing characteristics:
- Walk speed in meters per second on 20 meters (p 20 sp = 20/ p_s20m), m/s
- Walk speed in meters per second on 50 meters (p 50 sp = 50/ p_s50m), m/s
- Trot speed in meters per second on 20 meters (g 20 sp = 20/ g_s20m), m/s
- Trot speed in meters per second on 50 meters (g 50 sp = 50/ g_s50m), m/s
- Step length on 20 seconds for walk (p_ ss = p *20s/p* _20s), step/second
- Trot step length on 10 seconds for trot (g_ ss = p_10 s/g _10 s) step/second
- Step length in meters on 50 meters for walk (p _ long = p 50 sp/ p_ ss), meters
- Step length in meters on 50 meters for trot (g _ long = g 50 sp/ g_ ss), meters
- Walk speed at kilometers per hour on 50 meters (p50sp.sp=p50sp*3,6), km/h
- Trot speed at kilometers per hour on 50 meters (g50sp.gp=g50sp* 3,6), km/h
- wh (withers height) measured by ribbon
- cg (chest girth) measured by ribbon
- cbc (cannon bone circumference) measured by ribbon.

RESULTS AND DISCUSSION

By analyzing step characteristics (*Table 1*) we got the shortest time needed for one step per sec (0.97 s/s for stallions and 0.92 s/s for mares) in relation to literature and the top speed in meters per sec (1.65 m/s for stallions and 1.66 m/s for mares). Length of duration of a step for Lipizzaner mares was 0.92 step per sec and for stallions 0.97 step per sec. Length of duration of a trot step for Lipizzaner mares was 1.38 step per sec and for stallions 1.46 step per sec. Length of step on 50 meters for Lipizzaner mares was 1.78 meters and for stallions 1.69 meters. Length of trot step on 50 meters for Lipizzaner mares was 2.52 meters and for stallions 2.51 meters. Walk speed at kilometer per hour on 50 meters were 5.90 km/h for mares and stallions together. Average trot speed was 12.57 km/h for mares and 13.23 km/h for stallions.

Average walk step speed in meters per second on 20 meters for Lipizzaner mares was 1.66 m/sec, and 1.65 m/sec for stallions and together (*Table 1*). Average walk step speed in meters per second on 50 meters was 1.64 m/sec for all categories. With trained horses step speed is increased from gathered walk (1.37 m/s) to strengthened walk (1.82 m/s) with only slight increase of step frequency (*Barrey* 2001, cited from *Clayton* 1995). On Seoul Olympic Games average speed of strengthened walk was 1.88 m/s, step length was 1.95 m and duration was 1.03 s. These results are in accordance with our own research, although they are somewhat higher, which could be expected since these horses are elite Olympic trained horses. Walk step speed for Icelandic horses, Paso Finos, is between 1.7 and 2.3 m/s for walk, and their natural walk is something between walk, trot and gallop. That is the different way of pacing that consists of 4 symmetrical taps where legs aren't used diagonally but symmetrically.

Since the variance equivalence presumption of our research was wrong in two walk speed tests on 20 or 50 meters, indicated by F values (0.04 and 0.011), latitude level was corrected according to *Satterthwait* (1946). Variances of other studied characteristics were homogeneous. No statistical significant differences ($P>0.05$) were determined between other studied characteristics. Testing with t-test determined highly significant differences ($P<0.001$) for characteristics such as step length on 20 seconds for walk, step length on 10 seconds for trot, step length on 50 meters for walk and trot, while differences for other characteristics are not significant.

Table 1

Average value of speed and lenght of walk step and trot step for Lippizaner mares (n=53), stallions (n=18) and together (n=71)

Characteristic	MARES (n=53)				
	$\bar{x}$	S	Cv	min.	max.
p20sp	1.66	0.14	8.76	1.02	1.90
p50sp	1.64	0.13	8.04	1.07	1.91
g20sp	3.50	0.50	14.34	2.62	4.94
g50sp	3.49	0.50	14.21	2.61	4.98
p-ks	0.92	0.06	6.85	0.76	1.04
g-ks	1.38	0.09	6.43	1.24	1.67
p-long	1.78	0.15	8.19	1.08	1.99
g-long	2.52	0.26	10.22	1.86	3.24
p50spsp	5.90	0.47	8.04	3.84	6.90
g50spgp	12.57	1.79	14.21	9.40	17.93
Wh	165.77	3.98	2.40	157	172
Cg	190.60	6.72	3.52	178	206
Cbc	20.61	0.82	3.97	18	22
	STALLIONS (n=18)				
p20sp	1.65	0.09	5.56	1.44	1.79
p50sp	1.64	0.07	4.52	1.45	1.76
g20sp	3.64	0.58	15.81	2.51	5.02
g50sp	3.67	0.52	14.06	2.76	4.98
p-ks	0.97	0.07	7.23	0.87	1.11
g-ks	1.46	0.10	6.59	1.34	1.75
p-long	1.69	0.13	7.49	1.46	1.86
g-long	2.51	0.30	11.92	2.01	3.32
p50spsp	5.90	0.27	4.52	5.23	6.33
g50spgp	13.23	1.86	14.06	9.94	17.93
Wh	166.50	1.62	0.97	164	169
Cg	185.89	1.74	3.23	182	195
Cbc	20.55	0.51	2.49	19.5	21

Average trot step speed (*Table 1*) in meters/second on 20 meters for Lipizzaner mares in our own research was 3.50 m/sec, for stallions 3.64 m/sec and together 3.53 m/sec. Average trot step speed in meters / second on 50 meters for Lipizzaner mares was 3.49 m/sec, for stallions 3.67 m/sec and together 3.54 m/sec. Duration of the step was calculated by counting steps in 20 seconds. So the length of step for Lipizzaner mares was 0.92 steps/ second, for stallions 0.97 steps/ second and together 0.93 steps/ second. Duration of trot step was calculated by counting steps in trot in 10 seconds. So the length of trot step for Lipizzaner mares was 1.38 steps/ second, for stallions 1.46 steps/ second and together 1.40 steps/ seconds. The length of step on 50 meters for Lipizzaner mares was 1.78 meters, for stallions 1.69 meters and together 1.76 steps/seconds. Trot step length on 50 meters for Lipizzaner mares was 2.52 meters, for stallions 2.51 meters, as well as average trot step length for mares and stallions together. Walk speed at kilometre per hour on 50 meters is 5.90 km/h for mare, stallions and together. Average trot speed was 12.57 km/h for mares, 13.23 km/h for stallions and 12.74 km/h together. All head were measured by ribbon at the time of recording the steps. The average withers height of Lipizzaner mares was 165.77 cm, for stallions 166.50 and total 165.96 cm. Chest girth of Lipizzaner mares was 190.60 cm, for stallions 185.89 cm and together 189.41 cm. Cannon bone circumference of Lipizzaner mares was 20.61, for stallions 20.55 and together 20.60 cm.

The first research of length and speed of the step of Lipizzaner breed in Croatia was conducted by *Romić* (1940). After that step research was conducted by *Ogrizek* and *Sabadoš* (1943) and *Car* (1950). In his dissertation *Ljubešić* (1981) analyses the step length, duration of one step in seconds and step speed (min). The research results of states authors are presented in the *Table 2*.

Table 2

Comparative results of steps characteristics (*Ljubešić*, 1981) in relation to our own research

	Ogriz-Sab		Car	Romić	Ljubešić		Baban et al.	
	m	F			r	nr	m	f
Step length	1.66	1.61	1.57	1.72	1.69	1.82	1.69	1.78
Time (1s/s)	1.09	1.10	1.00	1.03	1.12	1.18	0.97	0.92
Speed (m/s)	0.92	0.88	0.92	1.01	0.91	0.93	1.65	1.66
Withers height	150	-	148	154	148	146	166	166

m: male, f: female, r: registered head, n: non registered head.

Comparing the data by the mentioned authors it can be noticed that non registered head according to *Ljubešić* (1981) had the longest step, followed by the step length of Lipizzaner mares from our own research. *Ljubešić* (1981) got the same value (1.69 m) for the step length of stallions from our own research for the registered head. Lipizzaner mares from our own research again had the shortest time needed for one step per second, while non registered mares had the longest according to *Ljubešić* (1981), which is understandable since the time needed for one step is the longest for this group. Top speed in meters per second was again accomplished by the Lipizzaner mares from our own research. As it can be seen from *Table 2*, there is a great difference in withers height of Đakovo Lipizzaner as opposed to the results got by the mentioned authors, which proves that the breeding goal for Đakovo Lipizzaner has been accomplished and that resulted in the expected values for the step length and speed. Since the mentioned authors did not conduct tests of trot of Lipizzaner, the values from our own research could not be compared to those of the other authors.

As *Ogrizek* and *Hrasnica* (1952) state, a step has to be rich, quick and appropriately high. The richness of the step is seen in its length. This is usually observed by the tracks of horse's last legs that have to be apart at least 10–30 cm from front leg of the same side. It is considered that a step is good if its length equals at least the withers height. Time for one step/second is extremely delicate to measure, because a step depends on the calmness of the head, on its temperament, how used to the step it is, and also on the guide. Differences between step length and withers height calculated from the authors' results and our own research are shown in *Table 3*.

Table 3

Differences between step length and crest height (*Ljubešić* 1981) in relation to our own research

Authors	Crest height	Step length	Difference (cm)
Ogrizek-Sabadoš	149.60	163.78	14.18
Car	148.43	156.60	8.17
Romić	154.29	172.40	18.11
Ljubešić-registered	148.01	168.91	20.90
Baban et al.	165.96	176.00	10.04

It can be seen from *Table 3* that *Lipizzaners* according to *Car* (1950) had the least difference between step length and crest height, followed by Lipizzaner stallions and mares (together) from our own research. If we compare only the registered Lipizzaner head, which is our goal, the largest withers height and the longest step were gained in our own research.

From trot on hand to speed passage (-2.18 m/s), step length has been reduced while the time between steps (+0.279) and diagonal setting have increased (+9.7 ms). Step length and its duration with standard bred breeds reaches the speed between 11.5 and 14.5 m/s. Trot speed is primarily increased as the result of increasing of step length with minimum change in step frequency (*Barrey*, 2001 as cited from *Wilson*, 1988).

The finalists in equestrian dressage on the Olympic Games in Barcelona showed the differences between temporal variables and gathered trot, passage and piaffe (*Barrey*, 2001 as cited from *Clayton*, 1997). The final gap is longer for piaffe (1.08 s) and passage (1.09 s) than for gathered trot (0.84 s), which means that piaffe and passage have lower step frequency. With trotters the trot step is so lengthened that it reaches maximum speed of 14.2 m/s with maximum step frequency of 2.52 step/s and maximum step length of 5.92 m (*Barrey*, 2001 as cited from *Barrey* and *associates*, 1995). On Seoul Olympic Games trot speed was being increased (7.03 m/s) and it was significantly higher than the one recorded on weaker national competition (5.97 m/s). The difference is primarily the result of longer step (4.15 m compared to 3.47 m) which is combined with somewhat slower frequency of long steps (101 step/min compared to 105 step/min) on Olympic Games.

CONCLUSION

Measuring step length and speed in both walk and trot on hand was conducted by digital video camera in Croatia for the first time, and the results are in concordance with the literature. Thus it can be concluded that the use of digital video camera and quantitative measurement enables the documenting of large number of outer body structure characteristics. The technique is very useful for studying the characteristics of steps. The highest withers height and the longest step were gained through our own research. This is a very valuable indicator since larger frame and longer step have been the breeding goal for years for Đakovo Lipizzaner. It is known that the training Lipizzaners had a high but short step which is inappropriate for top-level results in demanding driving sport. The selective work should be oriented towards the improvement of Lipizzaner step length since that is exactly the characteristic, along with richness, that is in great demand today for better achieving better results in driving sport.

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Determination of PPARGC1 Cys430Ser polymorphism and MHS genotype in Croatian autochthonous pig breeds

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ABSTRACT

The aim of the research was to determine PPARGC1 and MHS genetic status of two Croatian autochthonous pig breeds. The research was carried out on 20 Black Slavonian pigs and 20 Turopolje pigs. Genomic DNA was extracted from the blood. PCR was performed and PCR products genotyped by Restriction Fragment Length Polymorphism (RFLP). Allele frequency for PPRGC1 gene was determined by the Hardy-Weinberg equilibrium principle. Research results showed that A allele frequency in Black Slavonian pig was 44.44%, and of the T allele 55.56%. Homozygous genotypes (Ser/Ser) were dominant in Turopolje pig breeds (A=100.00%). Determined MHS status proved both pig breeds to be completely MHS resistant. Our results point out the necessity to further investigate genetic status of both pig breeds in order to improve constitution and quality traits of muscle tissue in pure pig breeds.
(Keywords: PPARGC1, MHS, Black Slavonian pig, Turopolje pig, allele frequency)

INTRODUCTION

Croatia is a home of two autochthonous pig breeds, of Black Slavonian pig and Turopolje pig. The main characteristics of this two breeds are late maturity and high fat content. They were of importance for local economy in the past. However, as the production focused on lean pig breeds, these two breeds gradually lost on their importance to become endangered and consequently included in the Endangered Breeds Protection Program of Central and Eastern Europe. Previous researches on these pig breeds focused primarily on determination of their productivity and slaughtering traits, the results of which pointed out excellent quality of muscle tissue of both Black Slavonian (*Kralik et al*., 1988; *Petričević et al*., 1988) and Turopolje breed (*Đikić et al*., 2002). It is expected that in the near future the two pig breeds will gain on their relevance in the improvement of constitution and quality of muscle tissue in pure pig breeds.

Intensive selection of lean pig breeds resulted in poor muscle tissue quality traits of some breeds. Scientific journals published numerous candidate genes for production traits or just molecular markers related to phenotypic diversity (*Houde, et al*., 2001). Peroxime Proliferator-Activated Receptor-Gamma Coactivator-1 (PPArGC1) and Malignant Hyperthermia Syndrome (MHS) genes are candidate genes for fattening and quality traits of muscle tissue. Recently, a few studies was carried out on PPARGC1 gene in pigs, because of its central role in adypocite differentiation (*Lowel* and *Spiegelman*, 2000) and impact of fat deposition and distribution in pig's body. It is also plays an important role as a transcriptional coactivator and metabolism regulation

(*Puigserver* and *Spiegelman*, 2003). *Kunej et al*. (2005) determined differences between Chinese and Western pig breeds in the T allele frequency (a T/A substitution at position 1378 in the PPARGC1 gene causing a Cys430Ser amino acid substitution). The conclusion was made that these differences could be connected with differences in fat and muscle tissue deposition and distribution between Western and Chinese pig breeds. Further studies on PPARGC1 gene are needed for better understanding of its role and function in order to determine its effect on fattening and slaughtering traits of lean pig breeds.

MHS gene (RYR1 gene) affects stress susceptibility in pigs mutation of that gene occurs at the nucleotide position 1843, resulting in cytosine/thymine substitution (*Fuji et al*., 1991). Mutation of that gene is common in lean pig populations and causes disturbance of muscle tissue quality. Despite of its negative effects, it is still one of the most important candidate genes in determination of slaughtering traits of carcasses and quality of muscle tissue. Many authors found out that MHS-carriers (recessive homozygotes) had more muscle tissue in carcass (*Aalhus et al*., 1991; *Pommier et al*., 1992; *Rosner et al*., 2003), but these traits were clearly related to stress susceptibility of pigs causing poor quality of meat (*Denborough*, 1998; *Rűbensam*, 2000; *Houde et al*., 2001). Relation among MHS genotypes and good quality traits of carcasses can be important in improvement of those breeds which traits were disturbed during the course of intensive selection. The aim of this research was to determine PPARGC1 and MHS status of Black Slavonian and Turopolje pig, and to elaborate their possible exploitation in improvement of production and slaughtering traits of lean pig breeds.

MATERIALS AND METHODS

The research was carried out on 20 Black Slavonian and 20 Turopolje pigs. Blood samples were collected on family farms in Eastern Croatia and in the area of Turopolje countryside. Genomic DNA was extracted from blood samples using standard phenol-chloroform-isoamyl alcohol (25:24:21) extraction protocol (*Ausubel et al*., 2000). Polymerase chain reaction (PCR) for PPARGC1 gene was carried out in a total volume of 20 µl containing 100 ng of genomic DNA, 1 x PCR buffer, 200 µM dNTP, 1 mM $MgCl_2$, 1 U Taq DNA polymerase (Fermentas, Litva) and 5 pmol of each oligonucleotide. The sequences of the oligonucleotides, covering 200 bp of the exon 8 central region, were as follows: PGC1-SSCP.F (5'-TAAAGATGCCGCCTCTGACT-3') and PGC1-SSCP.R (5'-CTGCTTCGTCGTCAAAAACA-3'). PCR was performed under following conditions: initial denaturation at 95 °C for 5 minutes, followed by 31 cycles of denaturation at 95 °C (40 s), annealing at 59 °C (40 s) and extension at 72 °C (50 s) with final extension step at 72 °C for 7 min. PCR products were checked on 2% agarose gel.

Digestion of PCR products was carried out in a final volume of 20 µl containing 13 µl of PCR product, 1x reaction buffer and 1 U of *Alu*I restriction enzyme (Fermentas, Lithuania). Reactions were incubated at 37 °C for 2 h and resolved on a 3% agarose gel.

Sequencing of PCR products was performed on the ABI sequencing machine (Perkin Elmer). Sequences were aligned in ClustalW and checked for A/T polymorphism at nucleotide position 1378.

PCR for MHS gene was carried out in a total volume of 20 µl containing 100 ng of genomic DNA, 1 x PCR buffer, 200 µM dNTP, 1 mM $MgCl_2$, 1 U Taq DNA polymerase (Fermentas, Lithuania) and 10 pmol of each primer. Primer sequences were as follows: RYR1.F (5'-CTGGTGACATAGTTGATGAGGTTTG-3') and RYR1.R (5'-

GTGCTGGATGTCCTGTGTTCCCT-3'). PCR was performed according to the following protocol: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C (1 min), annealing at 56 °C (1 min) extension at 72 °C (1 min) with final extension step at 72 °C for 2 min. PCR products were checked on 1.5% agarose gel. Restriction digestion of PCR products was performed with *Hin*6I (Fermentas, Lithuania) for 2 h at 37 °C and analyzed on 2.5% agarose gel. Allele frequency for each pig breed was calculated by the Hardy-Weinberg equilibrium principle, $p^2+2pq+q^2=100$. All analyses were carried out in the genetic laboratory of the Zootechnical department, Biotechnical Faculty in Ljubljana, Slovenia.

RESULTS AND DISCUSSION

The *Figure 1* presents restriction results of PPArGC1 gene by AluI restriction enzyme on 3% agarose gel. Animals homozygous for A allele (Ser) have 4 restriction sites resulting in 5 bands (61 bp, 60 bp, 31 bp, 27 bp and 21 bp). T allele homozygotes (Cys) have lost one restriction site resulting in 4 bands (121 bp, 31 bp, 27 bp and 21 bp). In heterozygous animals (AT), restriction digestion results in 6 bands. Seqence analysis of central region of the exon 8 PPARGC1 gene in Black Slavonian and Turopolje pig breeds exhibits nucleotide polymorphism at position 1378 (*Figure 2*).

RFLP analysis of PPARGC1 gene showed presence of only AA genotype in Turopolje pig (*Table 1*). This could be consequence of high proportion of inbreeding in this population of pig. Allele frequencies of PPARGC1 gene in Turopolje breed do not correspond with results obtained by *Kunej et al*. (2005) that reported the T allele to be dominant in fatty pig breeds, and the A allele to be dominant in lean breeds. The A allele frequency of Black Slavonian pig was 44.44%, and the T allele 55.56%.

Figure 1

PCR-RFLP fragments of T1378A polymorphism in PPARGC1 gene

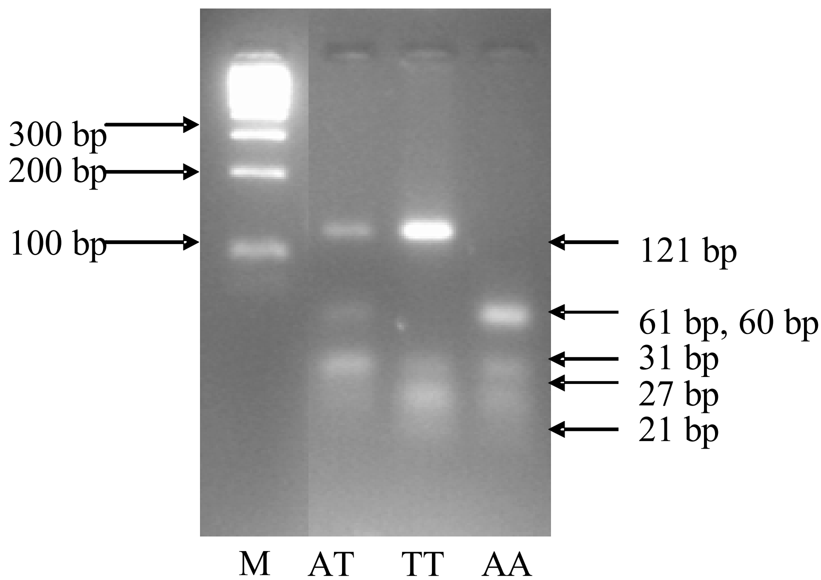


Figure 2

Nucleotide polymorphism at position 1378 in the central region of the exon 8

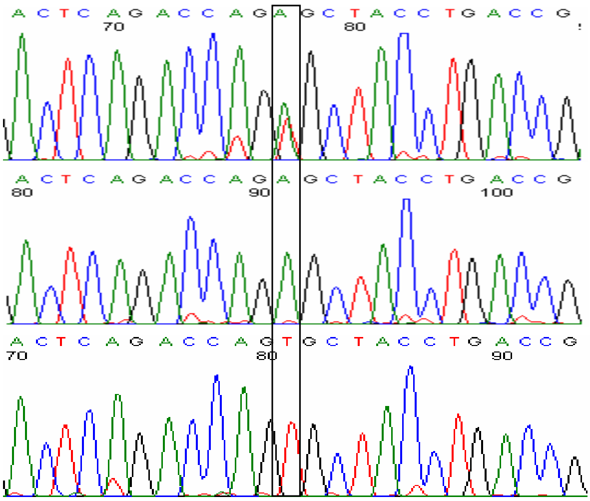


Table 1

Allele frequency of PPARGC1 gene in Black Slavonian and Turopolje pig

Pig breed	Cys430Ser genotypes				Allele frequency[2]	
	n	AA[1]	AT[1]	TT[1]	A	T
Black Slavonian	20	5	7	8	44.44	55.56
Turopolje	20	20	-	-	100.00	-

[1]Genotype AA:Ser/Ser, AT:Ser/Cys, TT:Cys/Cys.
[2]Allele frequency calculated according to Hardy-Weinberg equilibrium principle.

Figure 3

PCR-RFLP fragments of PPARGC1 gene in Turopolje breed

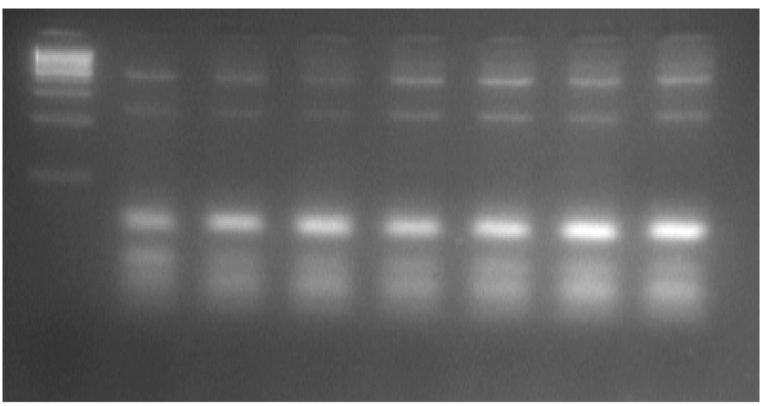
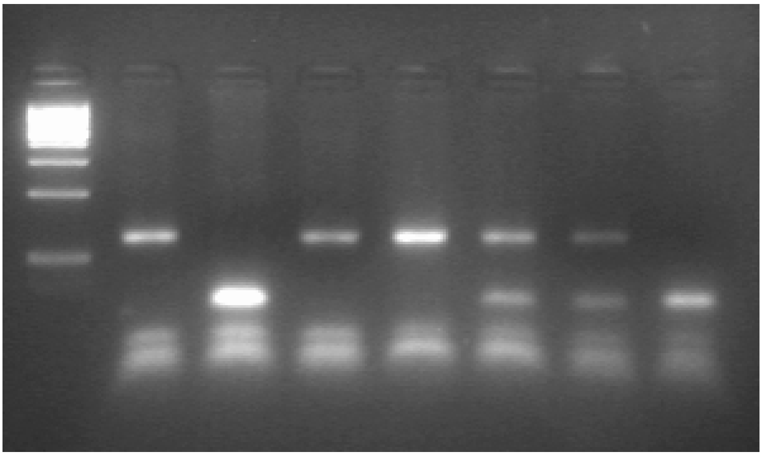


Figure 4

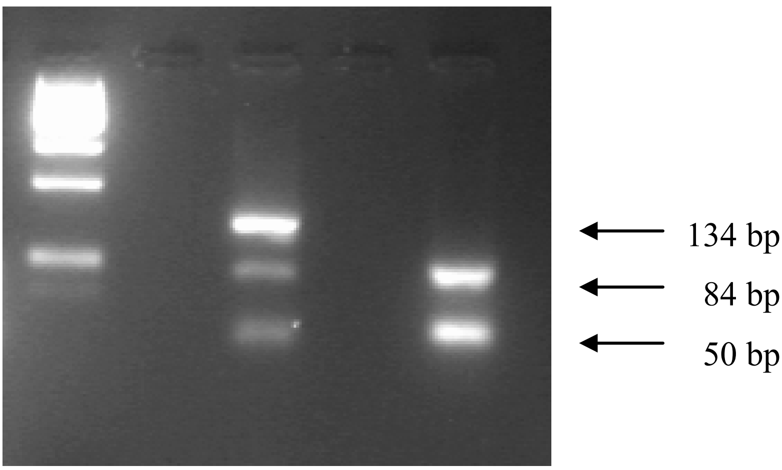
PCR-RFLP fragments of PPARGC1 gene in Black Slavonian breed



Restriction digestion of the MHS gene with Hin6I restriction enzyme results in two bands (84 bp and 50 bp) in homozygous animals. Recessive homozygotes exhibit only one band (134 bp), while in heterozygotes, digestion results in 3 bands on gel (134 bp, 84 bp and 50 bp). Restriction results in this research proved that pigs of Black Slavonian and Turopolje breeds were completely MHS resistant (*Figure 3*). Very good quality traits of meat, as well as the fact that pigs of Black Slavonian and Turopolje breeds are MHS resistant, points out the need to carry out more detailed research into genetic status of these breeds in order to determine their role in improvement of constitution and quality of muscle tissue in pure pig breeds in the near future.

Figure 5

PCR-RFLP fragments in MHS gene



CONCLUSION

Restriction analysis of PPARGC1 gene showed that homozygous genotypes (Ser/Ser) were dominant in Turopolje pig breeds (A=100.00%). The A allele frequency of Black Slavonian pig is 44.44%, and the T allele 55.56%

Determined MHS status of Black Slavonian and Turopolje pigs proved their complete resistance to stress, which points out their possible importance in improvement of quality traits of muscle tissue in lean pig breeds.

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Separation and determination of the tryptophan enantiomers

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ABSTRACT

Diastereoisomers of L- and D-tryptophan were formed with a chiral reagent 1-thio-β-D-glucose tetraacetate and o-phthaldialdehyde and they were separated from the derivatives of the other amino acids that occur in food proteins on an achiral column by high performance liquid chromatography. Mercaptoethanesulfonic acid that is an adequate agent for hydrolyzing proteins made the derivatization with o-phthaldialdehyde and 1-thio-β-D-glucose tetraacetate impossible, contrary the reaction completed in the presence of p-toluenesulfonic acid, but the oxidative losses during hydrolysis is significant. During boiling, the racemization of tryptophan can be detected above pH=9 after 12 hours, but the rate of conversion was lower than expected (<1%). The concentration decrease of L-tryptophan after 24 h was 2–5% depending on pH. Besides racemization other reactions e.g. oxidative deterioration may played a role in the loss of L- tryptophan.
(Keywords: racemization, tryptophan, mercaptoethanesulfonic acid, *p*-toluenesulfonic acid)

INTRODUCTION

The knowledge of the exact amino acid demand of the animals and the available amino acid content of the fodder is becoming a crucial point in animal nutrition due to its economical and environmental aspects. In the case of the domestic animal species the digestibility and the bioavailability of the D-enantiomer of a given amino acid is usually lower than that of the L-enantiomer. With the knowledge of the ratio of the amino acid enantiomers within the proteins of fodder, the amino acid requirements of the animals could be better satisfied. That could be especially important for essential amino acids like tryptophan (Trp). The determination of the amino acid enantiomers is also a question of importance in the human nutrition when the health effects due to the consumption of D-amino acids are studied.

In order to determine the amount of Trp in food samples the hydrolysis of the proteins is necessary. Under the most often used acidic hydrolysis conditions (6 M hydrochloric acid, 110 ºC, 24 h) the amount of this amino acid partially decompose due to oxidative processes and the loss is even higher in real food samples when carbohydrates are present. Hydrolysis in alkaline solutions (e. g sodium hydroxide) has been reported to preserve almost the whole Trp content of the sample (*Shizuko-Isole*, 1959; *Dréze*, 1960). Later collaborative studies clarified that the use of an internal standard is important in order to avoid the underestimation of the amount of Trp (*Landry* and *Delhaye*, 1994). The AOAC method uses NaOH hydrolysis method (*AOAC*) if the ratio of the enantiomers is not in the scope of the interest. In the case of the determination of D- and L-Trp, the enhanced degree of the racemization of Trp under alkaline conditions theoretically excludes this sort of solution for hydrolysis. Among acidic hydrolysis methods the highest recoveries were reported when 3 M mercaptoethanesulfonic acid (*Penke et al*., 1974) and 3 M *p*-toluenesulfonic acid containing 0.2% tryptamine (*Liu* and *Chang*, 1971) was used.

Besides the deliberation of the amino acids from the peptide chain the separation of the Trp enantiomers should be accomplished. In the case of high performance liquid chromatography (HPLC) there are three main possibilities: using columns with a chiral stationary phase, using an achiral stationary phase column with a chiral mobile phase, or derivatization with a chiral reagent and separation of the diastereoisomers formed on an achiral stationary phase column. Precolumn derivatization can be accomplished with *o*-phthaldialdehyde (OPA) and N-isobutyryl-L-cysteine (IBLC) (*Brückner* and *Westhauser*, 1994) or N-isobutyryl-D-cysteine (IBDC) (*Brückner et al*., 1995), with (+)-1-(9-fluorenyl)ethyl chloroformate (FLEC) (*Einarsson et al*., 1987), or with OPA and 1-thio-β-D-glucose tetraacetate (TATG) (*Einarsson et al*., 1987a).

The primary purpose of the research was to achieve an analytical method that renders practicable the detection of the racemization of Trp. First the separation of the Trp enantiomers in the form of diastereoisomers was accomplished on an achiral column in the presence of the other amino acids occurring in food proteins. Later on the applicability of acidic hydrolysis methods was investigated to clarify how they can be used prior to derivatization and analysis when protein bound amino acids has to be analyzed. Finally the racemization kinetics of free L-Trp of during heat treatments at different pH was studied.

Experimental

Derivatization and Analysis

Diastereoisomers were produced with OPA (o-phthalaldehyde) and TATG (1-thio-β-D-glucose tetraacetate) based on the methods of *Einarsson et al*. (1987a) and *Csapó et al*. (1995). 200 μL sodium-tetraborate buffer (pH=9.5) was added to 460 μL hydrolized protein solution or free amino acid standard solution containing 0.16 mg mL^{-1} amino acid, then 20 μL derivatization reagent (8 mg OPA and 44 mg TATG were dissolved in 1000 μL methanol) was added. The solution was mixed and after 6 minute-standing 20 μL was injected into the HPLC. OPA was obtained from Sigma Chemica Co. St Louis USA, and TATG was purchased from Aldrich-Chemie Gmbh, Steinheim, Germany. The separation was performed with a Superspher 60 RP-8e column or with a Purospher RP-18e 125×4 column (MERCK, Darmstadt, Germany); the temperature of the oven was 40 °C. The organic solvents were gradient grade methanol and acetonitril (MERCK, Darmstadt, Germany). The pH of the 39 mM phosphate buffer was set to 7.0 with 6 M hydrochloric acid solution, and ultrapure water for the preparation of the buffer was obtained from a Millipore Direct-Q instrument (Millipore, Molstein, France). The derivatives were detected with a fluorescence detector (ex.: 325 nm, em.: 420 nm). Derivatization and analysis were carried out with a MERCK-Hitachi HPLC containing the following modules: L-7250 programmable autosampler, L-7100 pump, L-7350 column thermostat, L-7480 fluorescence detector, AIA data conversion utility for D-7000 HPLC system manager (MERCK, Darmstadt, Germany).

Two sorts of amino acid standards were used for the HPLC method development. The first solution did not contain Trp. This solution consisted of 40 nmol mL^{-1} of the following protein constructed amino acids dissolved in water: D-Asp, L-Asp, D-Thr, L-Thr, D-Ser, L-Ser, D-Glu, L-Glu, D-Ala, L-Ala, D-Val, L-Val, D-Met, L-Met, L-D-, D-L-, L-L-, D-D-Ile, D-Leu, L-Leu, D-Tyr, L-Tyr, D-Phe, L-Phe, D-Lys, L-Lys, D-His, L-His, D-Arg, L-Arg and Gly. Secondary amino acids as Pro are not derivatized by OPA.

Cys is derivatized, but the fluorescence quantum yield is very low. The amide group of Asn and Gln hydrolyze during the hydrolysis of proteins and form Asp and Glu therefore these four amino acids were not included into the standard solution. The second standard solution consisted of 40 nmol mL^{-1} L-Trp and 20 nmol mL^{-1} D-Trp. The D- and L-amino acids were purchased from Sigma-Aldrich, St Louis, USA).

Hydrolysis

In order to eliminate the reaction of MES-OH and TATG three sorts of trials were applied. The aim was to remove or destroy MES-OH. First 5 cm^3 0.5 M $CuSO_4$ solution was added to 5 cm^3 mercaptoethanesulfonic acid solution (0.01 mmol D- and 0.01 mmol L-Trp and 4.5 mmol MES-OH) then the solution was centrifuged at 4000 g for 20 min. The pH of the supernatant was set between 5 and 6 with 4 M NaOH solution.

For the second time the mercaptoethanesulfonic acid solution (3 cm^3 3 M MES-OH and 0.01 mmol D- and 0.01 mmol L-Trp) was diluted three-fold, then 3 cm^3 oxidising solution (the mixture of one part of 30 (w/v)% H_2O_2 and nine part of 85 (w/v) % formic acid) was added to 1 cm^3 solution. The mixture was heated at 50°C for 5 minutes. After cooling the remaining performic acid was reacted with 0.52 g sodium-metabisulfite. The same procedure was accomplished with D- and L-alanine (0.01 mmol of each).

For the third time 1 cm^3 aliquots of mercaptoethanesulfonic acid solution (1 cm^3 3 M MES-OH solution and 0.01 mmol of D- and 0,01 mmol of L-Trp in 5 cm^3; 0.6 mmol MES-OH in each cm^3) was placed into a 25-cm^3-volumetric flask. The pH was set to 2, 6 or 9 with 4 M NaOH (a control sample also was prepared without pH setting) then 20 cm^3 distilled water and 1 cm^3 0.1138 g/cm^3 (0.612 mmol) iodoacetic acid solution was added and the volume was set with distilled water. Mercaptoethanesulfonic acid was obtained from Fluka Chemie Gmbh, Buchs, Switzerland.

In the case of *p*-toluenesulfonic acid hydrolysis 5cm^3 3 M *p*-toluenesulfonic acid solution containing 0.2% 3-(2-aminoethyl)indole (tryptamine) was added to the sample containing 15 mg protein. The hydrolysis was carried out in a closed ampoule under nitrogen at 110ºC for 24 h. Lyophilized sheep hemoglobin samples were hydrolyzed with this method. *p*-Toluenesulfonic acid was obtained from Sigma-Aldrich Chemie Gmbh, Steinheim, Germany. Neutralization was carried out with 4 M NaOH and the solution was diluted 125-fold with water.

Boiling

Free L-Trp-solutions (1 mg L-Trp/cm^3) with different pH values (pH = 3; 5; 7; 9; and 11) were prepared. The solutions were acidified with 6 M hydrochloric acid solution and the alkaline pH was set with 4 M NaOH solution. The ampoules with solutions were sealed after purging with nitrogen for 2 min. The L-Trp solutions were kept at 100±1 °C for 0; 5; 10; 20; 40; 60 min and 2; 4, 8; 12; 24; 48 hours.

RESULTS AND DISCUSSION

The separation of the derivatives of L- and D-Trp

Though the hydrolysis of proteins is prior to the analysis of the amino acids, the studying of the hydrolysis methods cannot be achieved without an analytical method for the separation of the Trp enantiomers. To reach the aim, D- and L- Trp were reacted with OPA and TATG and the resulting diastereoisomers were separated on an achiral stationary phase column following fluorescence detection with high performance liquid chromatography. An amino acid standard solution that contained the amino acids that are present in the food proteins with the exception of Trp and another standard solution of

D- and L-Trp was used for method development in order to avoid interferences.

During the optimization of the separation the sort of the stationary phase; the type (methanol, acetonitril or both) and the ratio of the organic solvent was changed. The best separation (see *Figure 1* for chromatogram containing both Trp and the other amino acids) was achieved on the RP-18 column and the composition of the mobile phase can be seen in *Table 1*.

Table 1

Eluent composition applied for the separation of OPA-TATG derivatives of L- and D-Trp from the derivatives of the other amino acids that occur in food proteins

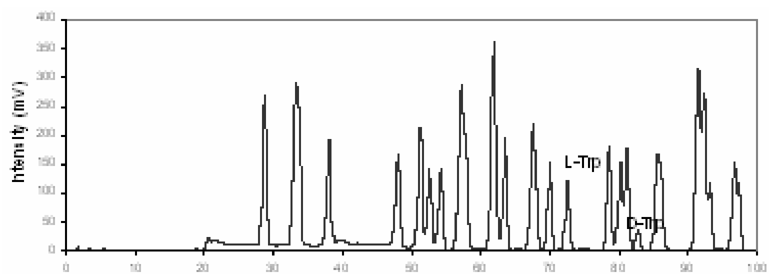
Time (min)	Metanol (*v/v*%)	Phosphate buffer (39 mM, pH=7.0) (*v/v*%)	Acetonitril (*v/v*%)
0	20	80	0
120	20	45	35
130	20	45	35
135	20	80	0
140	20	80	0

Column: Purospher RP-18e; 125 mm x 4 mm; flow rate: 1 cm^3 min^{-1}.

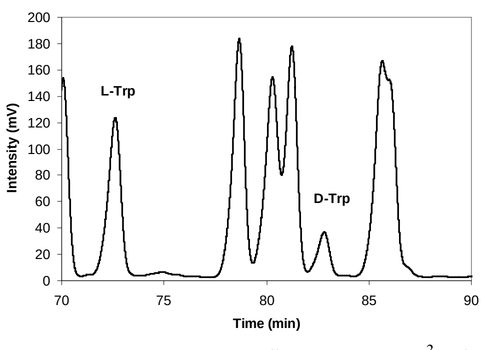
Figure 1

Separation of the OPA-TATG derivatives of L- and D-Trp from the derivatives of the other amino acids that are occur in food proteins
A=the complete chromatogram, B=part of the chromatogram

A



B



Column: Purospher RP-18e; 125 mm x 4 mm; flow rate: 1 cm^3 min^{-1}.

There was a significant difference between the fluorescence factors of the enantiomers. With the same concentration increase the relative fluorescence of L-Trp was 2.4-fold higher than that of D-Trp. The data of calibration graphs for the enantiomers can be seen in *Table 2*. The detector response was linear between 14 and 336 ng L-Trp/injection, and 14–700 ng D-Trp/injection. The limit of detection was 0.9 ng/injection for L- and 0.7 ng/injection for D-Trp. The limit of quantification for L- and D-Trp was 9.1 and 7.5 ng/injection, respectively. The relative standard deviation (RSD) in the low concentration range (14 ng L-Trp and 28 ng D-Trp/injection) were 1.1 and 4.1%; in the medium concentration range (140 ng L-Trp and 280 ng D-Trp/injection) were 6.8 and 1.9%; and in the high concentration range (276 ng L-Trp and 552 ng D-Trp/injection) were 1.6 and 2.3%, respectively. The number of replicates was 6.

Table 2

The calibration graph data of the OPA-TATG derivatives of the tryptophan enantiomers detected with a fluorescence detector (ex.: 325 nm, em.: 420 nm) (Number of calibration levels applied within the linear range: 9 for L-, and 10 for D-tryptophan)

Amino acid)	**Linear range nmol mL^{-1}**	**Correlation coefficient**	**Slope (A)** (x, y see below)	**Intercept(B)**	**LOQ nmol mL^{-1}**
L-Trp	3 - 72	0.9998	12.6	-2.6	2.2
D-Trp	3 - 150	0.9996	5.3	-3.0	1.8

x: height of the peak (mV)/concentration (nmol mL^{-1}).
y: concentration (nmol mL^{-1}).

Investigation of hydrolysis methods

Before the analyses of real samples different hydrolysis methods were tried out with standard Trp-solutions in order to examine if the following step the derivatization with OPA-TATG can be carried out.

Mercaptoethanesulfonic acid hydrolysis

In the presence of mercaptoethanesulfonic acid (MES-OH) D- and L-Trp did not form derivatives with OPA and TATG. Probably, instead of the bulky molecule of TATG, the MES-OH molecule reacts with OPA and the amino acid, and an achiral derivative of Trp forms. In the case of the other amino acids that occur in the protein MES-OH was applied for both hydrolysis and formation of achiral derivatives with OPA (*Csapó et al.*, 2004). In the case of Trp OPA/MES-OH derivatives can also be formed and used for the determination of the amount of (L+D) Trp.

When the knowledge of the ratio of the Trp enantiomers is important, the building of MES-OH into the derivative should be eliminated. When hydrolysis is completed and the solution with free Trp is ready to derivatization, MES-OH should be converted into nonactive form with respect to the above-mentioned derivatization. To reach the aim three trials were conducted:

Elimination of MES-OH in the form of metal salt: The copper-mercaptide precipitate was separated from the suspensoid with centrifugation and the clear supernatant was used for derivatization.

Performic acid oxidation of MES-OH: The thiol group of MES-OH was converted into sulfonic acid group. The remaining amount of performic acid was reacted with sodium-metabisulfite.

Reaction of MES-OH with iodoacetic acid: In order to block the thiol group, carboxyl-methyl derivative of MES-OH was formed.

In the first and the second cases we cannot detect OPA-TATG derivatives of D- and L-Trp. In the third trial there were some formation of the required derivatives, but the conversion rate of D-and L-Trp were poor. The amount of TATG was increased in order to provide enough TATG for derivatization, because TATG can also react with the remaining iodoacetic acid, but the level of conversion did not increase.

p-Toluenesulfonic acid hydrolysis

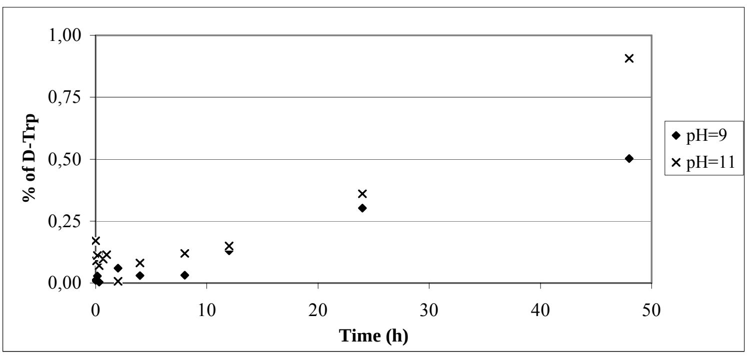
First *p*-toluenesulfonic acid was added to standard solutions of L- and D-Trp and it can be ascertained that in the presence of *p*-toluenesulfonic acid the reaction of D- and L-Trp into OPA-TATG derivatives was completed. When samples are heat-treated during hydrolysis the addition of tryptamine is necessary (*Liu* and *Chang*, 1971) because this molecule is a protecting agent again oxidative conversion of Trp. In the presence of 0.2% tryptamine in the *p*-toluenesulfonic acid solution the derivatization of Trp is blocked due to the amine group of tryptamine. The recovery of *p*-toluenesulfonic acid hydrolysis without tryptamine was reported to be low (*Liu* and *Chang*, 1971). The same tendency was observed when sheep hemoglobine was hydrolized without tryptamine and the recovery of L+D Trp was 44 ±2%. Despite of the low recovery the ratio of the L-and D-Trp can be established. The next step should be the use of another protecting agent such as 3-(3-indolyl)propionic acid that does not contain amine group but suitable for the protection of the indole ring of Trp from oxidative deterioration.

The influence of boiling on the Trp content in the function of pH and time

The loss of L-Trp due to racemization was not significant at lower pH values (pH=3−7). The amount of the D-Trp increases at pH=9 and pH=11 after twelve hours of boiling (*Figure 2*), but the rate of conversion (<1%) is lower than expected.

Figure 2

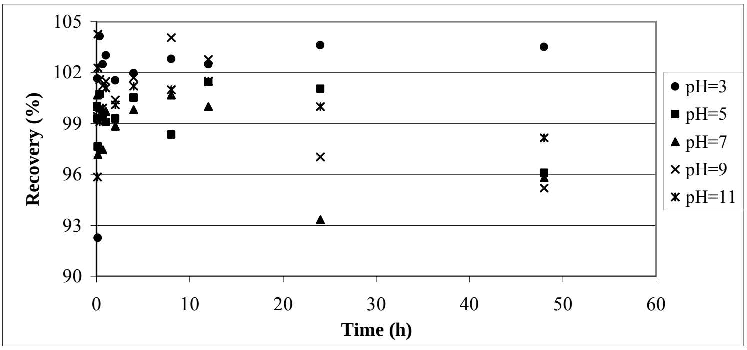
The increase of D-Trp content during boiling at 100 °C at pH=9 and pH=11 (% of Trp content)



After 24 h the amount of L-Trp tended to decline slightly (*Figure 3*). When the boiling exceeds one day, the loss of Trp can be 2−5%. Beside racemization other reactions e.g. oxidative deterioration of Trp indole-ring can be responsible for the loss of L-Trp.

Figure 3

Change in the L-Trp content during heat treatment at 100 °C in aquatic solutions at different pH



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Investigation of performic acid oxidation in case of thiol-containing amino acid enantiomers

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ABSTRACT

Performic acid oxidation of cysteine and methionine resulting in the formation of cysteic acid and methionine-sulphon has been applied in order to avoid the loss of these two sulphur containing amino acids during the acidic hydrolysis of proteins that is necessary prior to amino acid analysis. The aim of the research was assigned by the increasing demand for the determination of the amount amino acid enantiomers: the applicability of performic acid oxidation was evaluated in this point of view. Racemization of L-cysteine and L-methionine was found not significant during oxidation with performic acid, therefore this process can be applied before hydrolysis during quantification of cysteine and methionine enantiomers. Additionally, the quantification of cysteic acid and methionine-sulphon enantiomers was accomplished in the form of their diastereoisomer derivatives via the development of a reversed phase high performance liquid chromatography method.
(Keywords: performic acid oxidation, cysteine and methionine enantiomers, racemization)

INTRODUCTION

The determination of the amount of sulfur containing amino acids in foods and feeds involves some difficulties because under the generally used protein hydrolysis conditions (6 M hydrochloric acid solution, 110 ºC, 24 hours) a part of these amino acids undergo oxidative deterioration (*Martin* and *Synge*, 1945). In order to prevent these losses the thiol group of these amino acids was suggested to be converted into more stabile groups. With performic acid oxidation cysteine and methionine can form cysteic acid and methionine-sulphon (*Schram et al.*, 1954) and the loss of these molecules during hydrolysis is negligible related to that of the initial amino acids. This method has been used for decades for the determination of sulfur containing amino acids and cysteic acid can be analyzed rapidly by an ionic exchange liquid chromatography system (*Csapó*, 1982). Nowadays there is an increasing demand for the determination of the amount of the L- and D-enantiomers of the amino acids. The question arises if this sort of analysis needed to be operated whether the extent of racemization during performic acid oxidation is negligible or not. The purpose of the research was to investigate whether performic acid oxidation can be used when the aim is to determine the amount of methionine and cysteine enantiomers, and besides an RP-HPLC method was developed in order to separate the derivatives of these oxidized amino acids. In a preliminary research the separation of cysteic acid enantiomers has been accomplished (*Varga-Visi et al.*, 2000). In the present work the aim was to extend the separation and the investigation of performic acid oxidation to the other sulfur containing amino acid, methionine in order to determine the amount of methionine and cysteine in one single analysis.

MATERIALS AND METHODS

Oxidation with Performic Acid

A sample of cysteine and that of methionine (approx. 0.1 mM) was weighed into a vial. Five cm^3 performic acid, produced based on the method of *Hirs* (1956) was added and the mixture was heated at 50 °C for 15 minutes then it was cooled down immediately and lyophilized at -5 °C. If the sample contains only free amino acids the dried sample is washed with water into a 50 cm^3 volumetric flask. The pH was adjusted to 7 with 4 M sodium hydroxide, and the solution was ready for analysis.

Hydrolysis

For protein containing samples the oxidized and lyophilized sample was dissolved in hydrochloric acid (6 M; 5 cm^3) and hydrolyzed at 110 °C for 24 h. After cooling the solution was neutralized (pH 7) with sodium hydroxide solution (4 M).

Derivatization and Analysis

Diastereoisomers were produced with OPA (o-phthaldialdehyde) and TATG (1-thio-β-D-glucose tetraacetate) by the method of *Einarsson et al.* (1987). OPA and TATG were obtained from Sigma (St. Louis, MO, USA). The compounds were separated on a 125 mm×4 mm i.d. column packed with LiChrospher 100 RP-18. At the beginning of the experiments, the mobile phase consisted of 5% (v/v) tetrahydrofuran and 95% phosphate buffer (39 mM, pH=7.05), as in the case of the separation of OPA-TATG derivatives of cysteic acid enantiomers (*Varga-Visi et al.*, 2000). The temperature of the oven was 40 °C. The derivatives were detected with a fluorescence detector (λex 325nm, λem 420 nm). Derivatization and analysis were carried out with a MERCK-Hitachi HPLC comprising L-7250 programmable autosampler, L-7100 pump, L-7350 column thermostat, L-7480 fluorescence detector, and AIA data conversion utility for the D-7000 HPLC system manager. Reagents were pro analysis grade. Solvents (tetrahydrofuran and water) were HPLC gradient grade and purchased from MERCK (Darmstadt, Germany).

RESULTS AND DISCUSSION

Separation of the enantiomers of sulfur containing amino acids

The aim of the method development was to achieve an acceptable resolution within a reasonable range of retention factor k ($1<k<10$). The adequacy of resolution was the most important point of view of the method development because the amount of the D-enantiomer can be less with two orders of magnitude than the amount of L-enantiomer in foods and feeds. When the separation method of the OPA-TATG derivatives of D- and L-cysteic acid was developed, the type of organic solvent used, the organic solvent/buffer ratio and the stationary phase of the column were optimalized among analytical conditions. In order to separate the OPA-TATG derivatives of D- and L-methionine-sulphon the strength of the mobile phase had to be changed and some part of the resolution of the first diastereoisomer pair had to be sacrificed to elute the second diastereoisomer pair in time. Increasing the initial tetrahydrofuran volume ratio with only two percent (that means 7% tetrahydrofuran, 93% phosphate buffer) halved the retention of cysteic acid derivatives while the resolution also dropped significantly, from 2.1 to 1.4. But this value is still acceptable in case of diastereoisomer pairs. Separation of cysteic acid and methionine-sulphon derivatives in one analysis cannot be achieved using isocratic condition in this system thus a gradient program was developed. After an initial period when the cysteic acid derivatives were to be separated (0–14 minutes) the ratio of tetrahydrofuran was increased in the eluent. The changes of the resolution and the retention time of the methionine-sulphon derivatives in the function of the tetrahydrofuran–phosphate buffer composition of the mobile phase from the 20th minutes of analysis can be seen in *Table 1*.

Table 1

The influence of eluent composition on the resolution and retention time of OPA-TATG derivatives of methionine sulphon

Eluent composition % (v/v)		Retention time (min) OPA-TATG derivative of		Resolution
Tetrahydrofuran	Phosphate buffer (39 mM, pH=7.05)	L-methionine sulphon	D-methionine sulphon	
20	80	29.4	29.7	0.87
19	81	29.6	30.1	1.02
18	82	29.9	30.5	1.09
16	84	31.2	32.4	1.31

Fine-tuning of the tetrahydrofuran volume ratio from 20% (v/v) to 16% from the 20 min resulted in a retention increase of L- and D-methionine-sulphon to some extent but the resolution of these two peaks improved considerably (from 0.87 to 1.31).
The final mobile-phase gradient is given in *Table 2*.

Table 2

The mobile phase gradient for the separation of sulphur containing amino acids

Time (min)	Gradient composition (v/v%)	
	Phosphate buffer (39 mM, pH=7.05)	Tetrahydrofuran
0	93	7
13	93	7
14.5	85	15
20	84	16
31	84	16
35	60	40
45	60	40
47	93	7
50	93	7

The flow rate was 1 cm^3 min^{-1}.

With the use of the above gradient program the OPA-TATG derivatives of acidic amino acids can also be separated besides that of the sulfur containing amino acids (*Figure 1*).

Figure 1

Separation of OPA-TATG derivatives of cysteic acid, aspartic acid, glutamic acid and methionine-sulphon enantiomers with RP-HPLC (Resolution: D/L cysteic acid: 1.4; D/L methionine-sulphon: 1.3.)

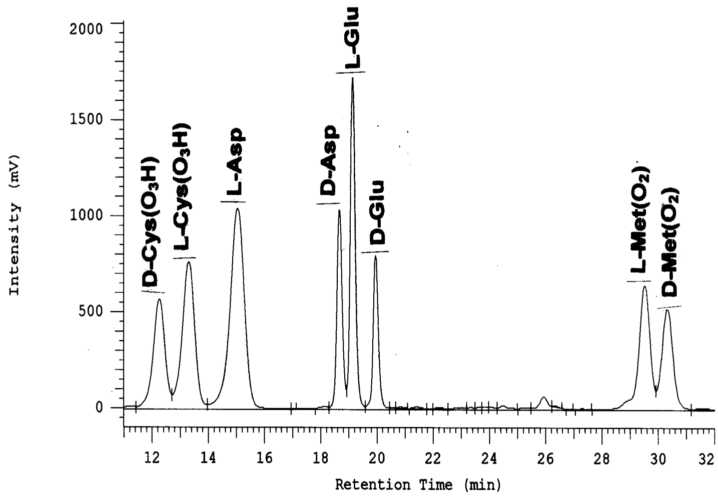
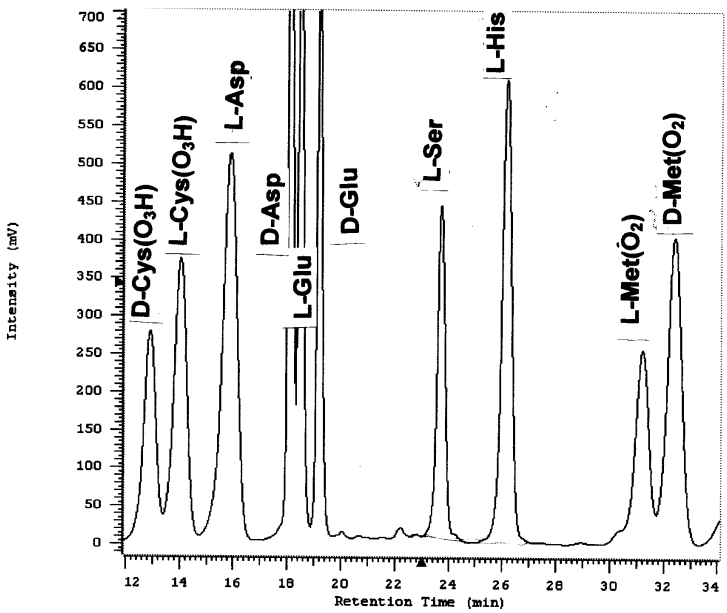


Figure 2

Separation of OPA-TATG derivatives of cysteic acid, aspartic acid, glutamic acid, and methionine-sulphon enantiomers, L-serine and L-histidine with PR-HPLC (Resolution: D/L cysteic acid: 1.4; D/L methionine-sulphon: 1.3.)



Based on the results of the preliminary research L-serine and L-hystidine derivatives were considered to elute in the same time period as the above amino acid derivatives therefore the possible interference was investigated. Derivatives of L-serine and L-hystidine were separated from the derivatives of the sulfur containing amino acids and separation was also acceptable for the derivatives of the acidic amino acids as *Figure 2.* shows. The detection limit for methionine-sulphon was 0.61 nmol/injection. The detector response was linear between 5.5 and 250 nmol/injection. At 50 nmol methionine-sulphon/injection the *RSD (n=3)* was calculated to be 8.5%.

Investigation of Performic Acid Oxidation

In case of cystein no significant racemization was observed during performic acid oxidation. For the other sulphur containing amino acid L-methionine standard of high optical purity was used to detect whether racemization occurred during oxidation. Solutions of L-metionine were oxidized like samples, and the quantity of D- and L-methionine-sulphon was measured. The D/(D+L)x100 ratio, corrected with the fluorescence factors of the corresponding OPA-TATG derivatives, proved to be less than 10^{-4} . This ratio is not significant when it is compared to the D/(D+L)x100 ratios occurs in food analysis, therefore it can be concluded that the extent of racemization of methionine during oxidation with performic acid is negligible.

To study the rate of conversion, that is the extent of the other losses during performic acid oxidation of the amino acid, L-methionine in standard solutions were oxidized and analyzed. The quantity of the product was determined by use of calibration curves of methionine-sulphon standard solutions. The rate of conversion from methionine to methionine-sulphon seemed to be higher than that of cysteine to cysteic acid (96±3% and 71±3% (n=3) respectively). Certainly the determination of the recovery needs to be accomplished separately in case of each substance under study.

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