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Reproduction as engine enhancing food security and sustainability

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Today's agriculture is faced with many "non-standard" impacts. Some of them have its demographical evidence, others are close to conspiracies. All of them are covered in to one topic "Food security and safety". Most often discussed topics are related to use of biotechnologies in reproduction and genetics, especially issue of GMO, use of genomics and request of sustainable development. Expected growth in global human population, recalculated so many times, but generally speaking, from present 6 billion to 8 bill. in 2020 or up to 9 bill. in 2050 (*United Nations. World Population Prospects*, 2002; *Nelson et al*., 2010) are emerging argument of increased intensity of agriculture production, ability to produce in less fertile areas, or in development (breeding) of species surviving in such areas even arid parts of the World. We can see increased demand for food sources in most rapidly growing regions. Even in Asia, part of the World's traditionally vegetable based diet, under social development, request for milk, milk products (butter, cheese, yogurt, ..), meat as well as cereals is rapidly increasing. Expected increase of meat production in USA and EU will sustain in present tendency, high increase is expected in China and "developing" world. To the 2020 meat consumption on China will reach 100 mill. ton an in developing world up to 200 mil. ton of meat. Same tendency is expected in milk (milk product) consumption with increase up to 150 mill. and 400 mill. ton in China and developing world, respectively (*UNDP*, 2006; *FAO* 2006).

From view of traditional breeding we can see higher importance of fertility of animal populations in present and their presence with higher economic value in selection indices. More than before reproduction technologies are discussed to help fulfill demand on food of animal origin. We know today, that orientation solely on production traits brought us in some breeds (populations) of reproduction los and significant economic expenses of restoring fertility. Insemination as first of broadly used and accepted biotechnologies helped us to fast forward with response in selection in conventional breeding, on the other hand having some inherent limitations due to increase of inbreeding and inability to isolate desirable and undesirable traits. We can state that embryo transfer, cloning or trans-genesis could solve present problems in reproduction and ensure production of animals for desired purposes. But then, specific aspect comes as important issue – public opinion or if you want consumer preferences. Issues of cloning and trans-genesis are in contradiction to clerical or publically accepted ethics.

This aspect is one of most important in present agriculture. Consumer doesn't see that in 1940 one farmer was able to feed only 19 people by its production ability. Today one farm produces food enough for 155 people. No one knows the story, that due to population growth, as well as energy demand and limitation in fossil sources, we produce enough food even using soils not suitable for other purposes (grain production), from materials not suitable for human nutrition, crop residues and by-products from

industry (fiber). These are result of selection, breeding and technological development. Public doesn't understand issues of culling as part of selection of animals better adapted to present technology, they don't understand uniqueness of rumen function and that feeding of by-products is safe and welcome in production process, because saving legumes and cereals, they don't understand that all this is made with one aim to feed growing global human population.

Consumers doesn't see these, they think we are on the wrong track, that produced food does contain suspicious ingredients, that due to mass production it is contaminated and even more that animals under conventional breeding are hurt. That we are wrong and only profit is of our interest. We are responsible for so called "civilization" as obesity and cardiovascular illnesses (*Edelman Insights*, 2012).

Positive influence of genetic on productivity is well proved. Today we don't doubt that phenotype is just result of genetic and environmental factors. We are aware of tools like animal recording, estimation of breeding values, selection tools and mating strategies to push desired population in direction of wish.

Many of the molecular genetic experiments have proved existence of favorable alleles inducing better results in reproduction of all farm species (*Trakovická et al.,* 2006). "Sires of today" (*Alta Genetics*, 2014; *Semex,* 2014) are genetically marked for favorable production traits (CheeseMakers, GenoMax) as well as reproduction traits (Concept+, Repromax, Immunity+). Challenge of today is to identify loci with effect of functional traits as birth difficulty, embryo survival (*Candrák et al.,* 2014) and others increasing longevity of individuals. These are the traits of interest today, because of economic impacts on agriculture branch effectiveness.

Strategic issues solved in our research are to develop tools for breeding value estimation in dairy cattle as well as tools for estimation of breeding values of functional length of production life. Tools and research is based on official milk recording system running in Slovakia under gesture of Breeding Services of Slovak Republic s. e. Those subjects have long, now we can say 20 years old history by us. Even not very well known, due to this we have been first country in the world (*Candrák et al*., 1997) using test-day animal model under commercial conditions. On the other hand, we are proud having people like G. Mészáros, who was developing tools in breeding value estimation of fLPL for population of Pinzgau cattle in Slovakia (*Mészáros et al*., 2008), than preparing and consulting of system for the whole dairy cattle population and finally future development of method to the full animal model (*Mészáros et al*., 2013). Length of productive life was analyzed by the use of methodology of the survival analysis. Studied fixed effects were the herd and year of calving, relative milk production, parity and stage of lactation, herd size change, age at first calving. The results for fixed effects in both models (sire vs. animal) were comparable. In separate models two genetic effects were considered: the sire of the cow and the animal itself with the corresponding pedigree records. The heritability estimates from the two models were different: h^2=0.08 for sire model and h^2=0.11 for the animal model. As the animal model accounts for all relationships in the population, including those between cows, it is the favorable alternative for a genetic evaluation. It is also a pre-requisite for a potential total merit index for Slovak Pinzgau cattle, where breeding values for functional length of productive life could play a decisive role when accounting for the functional traits. In present research accent is given to development of tools for other functional traits, mainly connected with reproduction.

In the field of diversity (sustainability) protection, we are not only concerning on traits of interest but also developing tools for monitoring and managing population for

future use. Intra-population diversity is an important part of the global diversity of farm animals. To prevent deterioration of genetic diversity, minimizing inbreeding in small populations is of prime importance.

Main cattle (Holstein, Simmental, Pinzgau) and horse (Hucul, Lipizan, Shagya-Arab, Slovak sport pony) breeds in Slovakia have their present status defined (*Kadlečík et al*., 2011, 2012; *Pjontek et al*., 2012; *Pavlík et al*., 2014). Determination of genetic variability based on pedigree information in Slovak Spotted breed was made based on pedigree completeness, characteristics based on probability of identity by descent and gene origin. The level of inbreeding it's gain per generation and relatedness were low, under 1%. The average value of relatedness coefficient in reference as well as in the whole pedigree file was higher than inbreeding coefficient. Therefore it is assumed that number of inbred individuals will increase in the next generation. The analysed horse populations consisted of 656 Hucul horses, 2052 Lipizan horses, 1951 Shagya Arabian horses and 220 Slovak Sport Ponies. The pedigree completeness of the reference population was evaluated for each animal based on the number of fully traced generations, the maximum number of generations traced and the equivalent complete generations. The equivalent complete generations ranged from 4.93, for the Slovak Sport Pony, to 10.25, for the Lipizan horses. The average value of inbreeding ranged from 2.67%, for the Slovak Sport Pony, to 6.26%, for the Hucul horses. The average relationship coefficients were from 3.08%, for the Shagya Arabians, to 9.34%, for the Huculs. Individual increases in inbreeding ranged from 0.43 %, for the Lipizans, to 1.06%, for the Huculs, while the realised effective sizes were from 117.14 to 47.67 animals. The analyzed populations were derived from 80 to 499 founders. The effective number of founders ranged from 26 to 160, while the effective number of ancestors was from 7 to 32. There is increased demand for sustain monitoring of populations of "genetic preserves". In particular, mating program with control of inbreeding in horses is under development in experimental horse population. Optimization of breeding plans according sustainability, includes systems of breeding value estimation, pedigree structure development as well as estimation of expected genetic improvement (*Kasarda et al*., 2004, 2007, 2014). It has been shown that increasing the number of sires to breed sires results in decreased response to selection in all alternatives. At the same number of sires a *MOET* schemes yield the highest gain but also highest inbreeding. When restricting the rate of inbreeding to a value between 0.5-1 % per generation the highest gain was obtained by using five progeny tested sires per year under young sires breeding scheme design. Observed was inbreeding depression -39.60 SKK (1€ = 30.128SKK) of SPI, -8.95 kg in EBV of milk, -0.37 kg in EBV of fat and -0.36 kg in EBV of protein, respectivelly. We simulate scenarios of possible development in population when constraining inbreeding further. In classical approach a maximum avoidance of inbreeding (MAI) mating strategy is used and compared with a random mating alternative. The parameters of the simulation were based on the structure the Slovak Pinzgau active population of 2868 animals (930 purebred cows). Simulated was selection under a total merit index (TMI) covering the milk production, functional LPL and the live weight breeding value estimation results. The heritability of TMI ($h^2 = 0.09$) was estimated using a REML single trait animal model. Alternatives were build respecting theoretical assumptions of a closed population structure, fixed number of mating per parent, and equal use of sires in insemination. Animals in generation 0 were set as founders without pedigree information. In separate simulation runs, the number of sires of sires varied with 40 dams of sires in all cases. The sex ratio of the offspring was assumed to be 50/50 male/female. Minimum ten (due to achievement of Bulmer

equilibrium) to twenty consecutive generations were simulated for both random and maximum avoidance of inbreeding. Simulation results showed that the use of a maximum avoidance of inbreeding mating strategy would lead to significantly decreased rates of inbreeding while maintaining suitable levels of genetic gain in the Slovak Pinzgau population.

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Male reproduction in the polluted environment – review

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ABSTRACT

Endocrine Disrupting Chemicals (EDC-s) present in the environment can cause disturbance in the reproductive biology of animals and humans. While most of the studies focus on the adverse effects on the hormonal system, recent findings indicate a direct effect on germ cells. The present review introduces laboratory assays used to study several structural and physiological aspects of spermatozoa exposed to EDC-s.
(Keywords: environment, endocrine disrupting chemicals, reproduction, sperm, flow cytometry)

ENDOCRINE DISRUPTORS IN THE ENVIRONMENT

There is a growing interest in the presence of Endrocrine Disrupting Chemicals (EDC-s) in the environment (*Mills and Chichester*, 2005). EDC-s can disturb the normal reproductive physiology of animals (and humans as well), including abnormalities in sexual development (like hermaphroditism), changes in the normal sexual behavior, etc. Well known examples of EDC-s are polychlorinated biphenyls (PCB), alkyl-phenols or ethinyl-estradiol, a compound in human contraceptives (*Larsson et al.,* 1999; *Waring and Harris*, 2005).

Several countries initiated research projects to study the presence of EDC-s in the environment as well as to reveal their biological effects (*Hutchinson et al.,* 2000; *Vethaak et al.,* 2002). Most of the studies focused on the adverse effect of EDC-s on the endocrine homeostasis, however, a recent study clearly demonstrated effects at a different level: EDC-s can directly disturb the normal function of spermatozoa at physiologically relevant levels in the body fluids (*Schiffer et al.,* 2014).

Spermatology assays, originally developed for the routine semen quality controls at artificial insemination centers or human infertility clinics can be directly applied to the study of the effects of EDC-s on sperm structure and function. Such up-to-date automatized laboratory methods like flow cytometry (*Spano and Evenson*, 1991; *Hossain et al.,* 2011) or Computer-Assisted Semen Analysis (CASA – *Vetter et al.,* 1998) are precise and accurate tools of studying the disturbing effects of EDC-s at the cellular or subcellular level.

At the cell analysis laboratory of the Georgikon Faculty of the University of Pannonia we are currently testing the applicability of several sperm function assays based on flow cytometry to study the effect of EDC-s, heavy metals and adverse environmental conditions on spermatozoa, with a special focus on fish sperm since the gametes of externally fertilizing species theoretically may suffer more damage than the cells of internally fertilizing species (currently we use boar sperm as model to test the effects of EDC-s on mammals, too). We have chosen several cytotoxic and genotoxic

end points to measure, including mitochondrial trans membrane potential, phospholipid asymmetry and intactness of the plasma membrane, oxidative DNA damage and DNA fragmentation. Cytotoxic effects may trigger an intracellular cascade of events: defective mitochondria are the main source of intracellular Reactive Oxygen Species (ROS) which induce plasma membrane lipid peroxidation as well as DNA damage (first oxidative damage then consequently the development of DNA strand breaks leading to DNA fragmentation (*Aitken et al.,* 2012).

FLOW CYTOMETRIC ASSAYS TO STUDY SPERM STRUCTURE AND FUNCTION

Plasma membrane integrity, mitochondrial activity

Spermatozoa can be divided into the following domains: head, midpiece and tail, and into subdomains within these regions. The different subdomains of the sperm head plasma membrane are involved in separate gamete interaction events (zona binding, acrosome reaction, zona penetration, fusion with the oolemma, etc.). The acrosome, a large vesicle on the apical part of the sperm head, contains the hydrolytic enzymes necessary for zona pellucida penetration. The midpiece contains mitochondria and is involved in energy production. The tail is involved in motility. Several flow cytometric assays have been developed for assessing the plasma membrane integrity of the head, the integrity of the acrosome or for evaluating mitochondrial function. However, there is no flow cytometric assay for the evaluation of the plasma membrane integrity of the tail domain. Earlier we showed with a light microscopic staining method that spermatozoa with intact head membrane but with disrupted tail membrane are not motile therefore they are functionally dead (*Nagy et al.,* 1999). To be able to measure the same attribute by flow cytometry, we currently develop and test a fluorescent staining method specific for the integrity of the sperm tail plasma membrane. In order to distinguish spermatozoa from non-sperm particles (especially in extended semen) we apply the LIVE/DEAD® Fixable Viability Kits from Molecular Probes (Eugene, OR, USA) which label the viable and dead cells with the same color but different intensity (*Nagy,* 2007). This would be advantageous in multicolor flow cytometry as only one detector is needed to detect live/dead status. Moreover as our preliminary experiments indicated, these kits are able to indicate membrane rupture on every sperm subdomain, including the flagellum, not only the head. As a logical extension of the multiparameter approach, a fluorescent staining for the assessment of the mitochondrial activity could be added.

We have developed and tested a more objective method of evaluating sperm quality than the current subjective motility evaluations by testing the applicability of a novel fluorescent probe, Mitotracker Deep Red 633 (M-22426), for measuring the mitochondrial activity of spermatozoa both after freezing/thawing and after swim-up selection, using flow cytometry. The proportion of spermatozoa with high mitochondrial activity as determined by Mitotracker Deep Red 633 showed a high correlation with sperm motility (*Hallap et al.,* 2005).

Capacitation, early membrane changes

The capacitation process of spermatozoa involves complex changes in the composition and orientation of molecules at the surface of the sperm cell. Capacitation is defined as a preparative step; a sperm cell must undergo a priming process before it can bind to the zona pellucida and initiate the acrosome reaction. Flow cytometric assays like the merocyanine 540 staining (*Hallap et al.,* 2006) or the Annexin V-FITC assay

(conventionally used in apoptosis studies) allow discrimination between sperm subpopulations that undergo the capacitation induced transitions and cells that do not respond to the induction. Cryopreservation induces similar membrane changes in the surviving, intact sperm cell population. Such spermatozoa have shortened lifespan, therefore it would be interesting to see individually different responses to the cryopreservation process and relate them to fertility differences. This physiological process is extremely important area to study from the point of view of actions of EDC-s on sperm, as recent findings indicate that several EDC-s induce capacitation-like events via activation the CATSPER membrane channels and affecting intracellular calcium levels (*Schiffer et al.,* 2014).

DNA damage, chromatin status

The structure of the sperm chromatin is unique among other cell types, as histones are replaced by transition proteins and finally by protamines during spermatogenesis, resulting in an extremely condensed DNA (*Dadoune*, 1995). Proper condensation stabilizes the DNA and makes it less sensitive to oxidative damage, however, mature spermatozoa are not able to repair DNA damage as they are transcriptionally inactive. Abnormalities of the sperm chromatin structure can cause reduced fertility, abnormal pronuclear formation or early embryo quality and pregnancy outcome (*Evenson*, 1999).

Studies on domestic mammal species indicated that the early embryonic death is often caused by the nuclear defects (DNA fragmentation) of the fertilizing spermatozoa (*Evenson*, 1999). DNA strand breaks in spermatozoa can be caused by oxidative stress, heat stress, radiation or protamine deficiency (*Varner*, 2008). Due to incomplete protamination spermatozoa will be less compact and consequently more sensitive to attack by endogenous or exogenous agents, like nucleases, free radicals or mutagens (*Oliva*, 2006). Spermatozoa carrying damaged DNA look normal with conventional laboratory tests, but may induce failure in embryonic development. As mature spermatozoa are transcriptionally inactive, DNA damage may not be expressed until mitosis at the time of spermatozoon-oocyte fusion (*Varner*, 2008). Environmental factors like chemicals, excessive temperature, air pollution can cause abnormal sperm chromatin integrity (*Evenson and Wixon*, 2005).

The “gold standard” method to measure the fragmentation rate of sperm DNA is the so-called Sperm Chromatin Structure Assay (*Evenson*, 1999; *Spano and Evenson*, 1991). The test measures the ratio of the intact, double-stranded DNA and the fragmented, single-stranded DNA using acridine orange staining and flow cytometry. Earlier stage of DNA damage due to ROS attack can be detected with the OxyDNA assay which identifies 8-oxoguanine, a DNA adduct (*Hoornsta et al.,* 2003).

CONCLUSIONS, FURTHER DIRECTIONS

We have tested the adverse effect of several EDC-s, including irgasan, a bactericide agent and cadmium, a heavy metal with endocrine disrupting capabilities on silver carp and boar spermatozoa. Our initial results indicate negative effects on mitochondrial membrane potential, plasma membrane phospholipid asymmetry, moreover, oxydative DNA damage was also observed. Based on these findings we believe that flow cytometry offers a unique tool to study subcellular effects of EDC-s on spermatozoa. We plan to extend the array of tests with measuring intracellular calcium and ROS levels as well as applying dynamic flow cytometry with time parameter analysis to reveal the kinetic changes in sperm cells exposed to EDC-s.

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Genetic aspects of fertility traits in dairy cattle – review

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INTRODUCTION

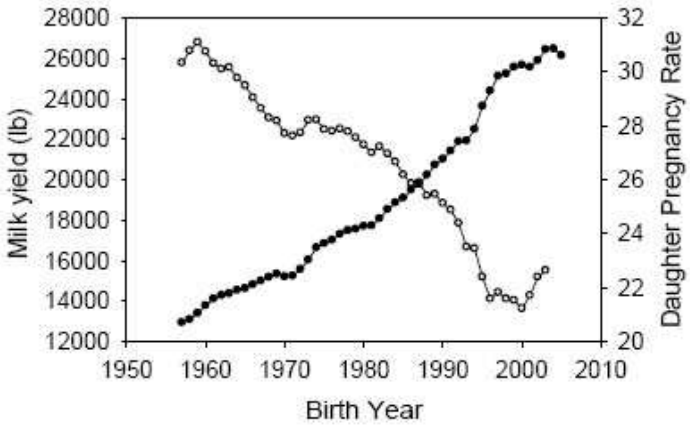
Fertility is considered a complex trait influenced by many physiological and disease-related variables. These is considerable scientific evidence to support the view that fertility performance is influenced by environment (E), genetics (G) and the interaction between G by E (GxE). Many hypothesis have been proposed to explain this including genetics, physiology, nutrition nad management, and these factors have been investigated at the animal, organ and celluar level at critical time points of the productive life of dairy cows (*Walsh et al.*, 2011).

It is largely known that, fertility in dairy cows strongly decreased over the last decades as milk production per cows has highly increased. Hence, the reproductive efficiency is became an high priority in all systems and it is considered higher in seasonal calving systems as the opportunity for cow to calve and become pregnant is time limited to ensure a calf per cow per year in synchrony with grass growth (*Dillon et al.,* 2006). Over the last 30 years, genetic selection for increased milk production, particularly within the North American Holstein-Friesian genotype, has been very successful. Between 1985 and 2003, the rate of phenotypic gain in milk production per cow per year has been 193 kg for the United States, 131 kg for the Netherlands, 35 kg for the New Zealand and 46 kg for Ireland (*Dillon et al.*, 2006). In Italy, the rate of the phenotypic gain in milk production per cow per year has been 112 kg in Holstein-Friesian with an average increase of the calving interval of 1.4 d per year (*Cassandro and Penasa*, 2010). Despite these countries having diverse production systems, genetic selection criteria and climatic conditions, they all report a sensible decline in reproductive performance during the same period inducing, in recent years, the emphasis within selection indices for Holstein-Friesian has shifted from predominantly production to funcional nonproduction traits associated with improved health and fertility (*Miglior et al.*, 2005).

Poor reproductive performace often leads to premature culling and decreased productive career of dairy cows. The association between the declines in fertility and milk production in the last decades, is evident in the Holstein population, as reported in *Figure 1* (*USDA-ARS AIPL*, 2007).

Figure 1

Trends in milk production and pregnancy rate by year of birth in the North American Holstein population (*USDA-ARS AIPL*, 2007)



However, there is now evidence that the phenotypic historical decline in fertility has reached a nadir and begun to improve "versus to zenit" (*Crowe*, 2008; *Norman et al.*, 2009).

Moreover, new research areas, as understanding genotype by environment interactions are crucial in determining the best health and management practices to achieve high levels of productive and reproductive efficiency. Recent studies have reported higher reproductive performance in high milk producing herds (herd average of > 10,000 kg milk production per lactation) than producing herds and concluded that this was likely due to better nutritional and reproductive management (*LeBlanc*, 2008).

Aim of this review, is primarily to review the effect and improving fertility through breeding strategies. This paper review the genetics aspects and strategies and their potential consequences affecting fertility in dairy cows.

DIRECT MEASUREMENT

An important direct measurnment of fertility is the pregnancy rate that measures how quickly cows become pregnant again after calving. It is defined as the percentange of nonpregnant cows that become pregnant during each 21-d period, because each eastrus cycle represents one chance for a cow to become pregnant. In recent years, many reproductive specialists have recommended this measure of reproductive success over the more traditional measure days open. Pregnancy rate calculations are more current, cows that do not become pregnant are included in calculations more easily, and larger rather than smaller values are desirable, simplifying selection by producers. Pregnancy rate can be calculated in function of voluntary waiting period and days open, as follow:

$$\text{Pregnancy rate} = 21/(\text{days open} - \text{voluntary waiting period} + 11)$$

where, voluntary waiting period is the initial phase of lactation during which no inseminations occur. The voluntary waiting period may vary across herds or seasons but would not affect genetic evaluations unless it differed for cows within the same herd-

year-season. The constant factor of 11 centers the measure of possible conception within each 21-d time period such that cows conceiving during the firt 21-d period receive 100% credit on average and so on. As an example (assuming a voluntary waiting period of 60 d), a herd that averages 154 d open has a pregnancy rate of 20% while a herd averaging 133 days open has a pregnancy rate of 25%. Across the possible range of days open, this formula produces far from linear results (*Figure 2*).

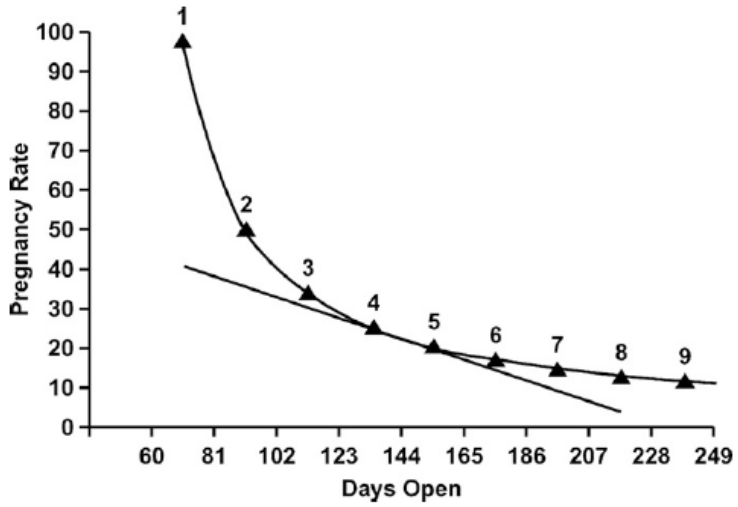
The genetic correlation between days open and pregnancy rate is estremely high (0.99) beacuse the only way to reduce days open is for cows to become pregnant at a faster rate. Knowing record on days open is it possible to transform data in pregnancy rate using this simple linear function, as reported by *VanRaden et al*. (2004):

$$\text{Pregnancy rate} = 0.25 \times (233 - \text{days open})$$

Reliable data on days open and on consequence on pregnancy rate is the most difficult aspect on using this direct measurment. Due to this, calving interval (CI), that is traditionally considered the main fertility indicator during the productive life of dairy cattle, might not be the most desirable direct measures of reproductive efficiency because of measures of CI are available only for cows that calve 2 or more times and not for females that do not calve and are culled. Moreover, CI is not an early measure of fertility and it is not an adequate selection tool for breeding organizations, which select bulls on the basis of the earliest information recorded on their female offspring. Consequently, indirect measurements of CI are more interesting for breeding programs based on improvement of fertility aspects.

Figure 2

Comparing nonlinear and linear trend from days open to pregnancy rate when a cow has one chance (numbered) to become pregnant during each 21-d cycle (*VanRaden et al.*, 2004)

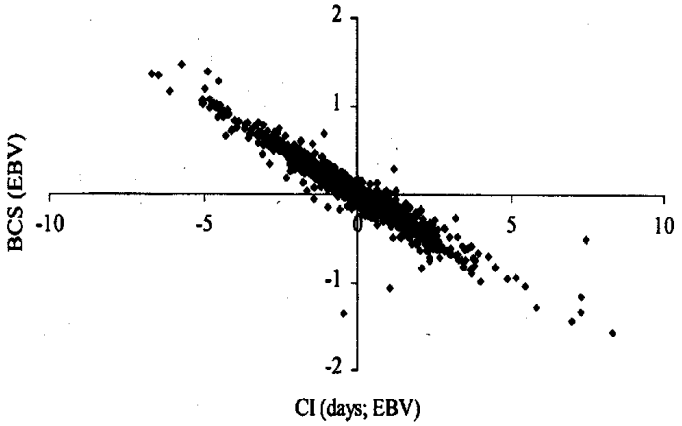


INDIRECT MEASUREMENT

One of the most interesting indirect measures of reproductive performance is the body condition score (BCS). Most studies on the relationships between BCS and fertility traits have been carried out (*Pryce et al*., 2000, 2001; *Dal Zotto et al*., 2007). Indeed, *Dal Zotto et al*. (2007), estimated a genetic correlation between BCS and CI of -0.35, indicating a moderately negative genetic association for these traits. *Pryce et al.* (2000), plotted BCS estimated breeding values versus CI estimated breeding values for 3,770 sires showing a strong linear realtionship (*Figure 3*). Therefore, cows that are thinner are more likely to have a longer CI. It is likely that cows are mobilizing body tissue to substain milk production, so BCS or BCS change is likely to be closely related to energy balance. Cows in negative energy balance, particularly in early lactation, may be yielding milk at the expense of reproduction. Hence, condition score has the potential to be used in breeding programmes. Genetic differences in the shape of the profile of depletion of reserves in early to peak lactation followed by recovery during the rest of the lactation may help to identify animals most suitable for improving fertility. Also, a flatter lactation curve may be a way of avoiding short-term nutrient deficits in dairy herds (*Pryce et al*., 2004).

Figure 3

Estimated breeding values (EBV) of BCS versus calving interval (CI) for sires obtained from univariate analyses (*Pryce et al.*, 2000)



As already mentioned, the fertility trait is very complex and it is for this reason that to improve the estimation accuracy of the index in Italy (*Biffani*, 2008) was proposed an aggregate index based on direct and indirect correlated traits. This approach not only allows to take into account the complexity of the fertility trait but also improves, increasing the reliability of the index. Since February 2006 for the Italian Holstein is available an aggregate index for fertility, whose goal is to improve the conception to first service. This index is obtained by analyzing multi-trait, ie including 3 directly traits, related to fertility (calving interval, calving-first insemination interval and non-return rate at 56 days), and 2 indirect traits (milk production and BCS). *Table 1* shows the 5 traits used and their relative weight in the index aggregate itself. The weights assigned to

each individual trait are not random, but depend on the genetic relationship between the traits themselves, and above all depend on the relationship that exists between them and the goal of the index: the conception rate at first service. Using these 5 traits there is an average increase of the reliability of almost 7%. The increase is mainly due to the greater amount of information available, but also to the contribution of two traits, milk and BCS, which have a heritability greater than that of the classic traits related to fertility.

Table 1

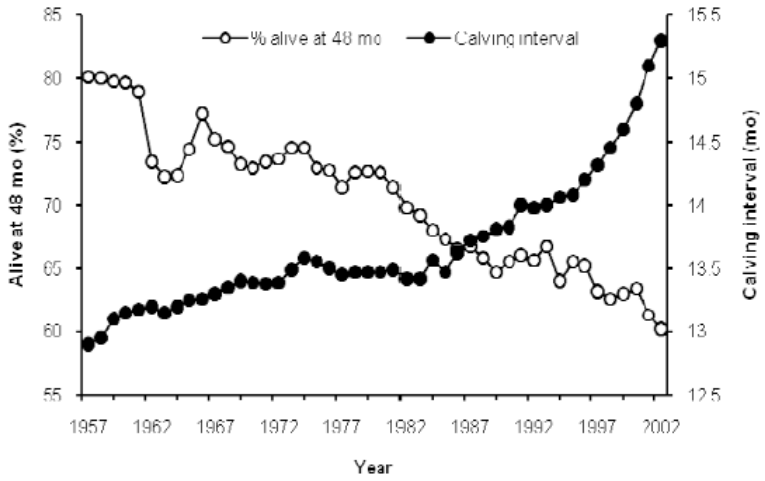
Traits included in the index aggregate fertility and their relative importance (*Biffani*, 2008)

Trait	Relative emphasis, %
Calving interval	51
Non return rate at 56d	17
Calving to 1st insemination	16
Milk yield, kg of 305d at mature equivalent	9
Body condition score	7

Moreover, improving fertility will also allow to avoid a reducing on longevity as reported by *Oltenacu and Broom* (2010). In *Figure 4* are shown the association between the declines in fertility, that reflected in increased calving interval, and decrease in longevity, measured by the proportion of cows still alive at 48 months of age (stayability) in Holstein cows in the north-eastern United States, from 1957 to 2002. Poor reproductive performance often leads to premature culling and decreased longevity of dairy cows.

Figure 4

Average calving interval and proportion of cow salive at 48 months of age over time for Holstein cows in the north-eastern United States (*Oltenacu and B*room, 2010)



GENETIC PARAMETERS

For many years, due to low heritability values, there was a perception that genetics could not contribute to the improvements in fertility traits. Therefore, if genetics contributed indirectly to deterioration in fertility, then genetics can also contribute to its improvement.

The heritability of traditional fertility measures across different countries and breeds of cattle tend to be less than 5% (*Pryce and Veerkamp*, 2001). In *Table 2* are shown the heritability values of different fertility traits used in different countries (www.interbull.org, 2014).

Table 2

Heritabilies of fertility traits used in different countries (www.interbull.org, 2014)

Country	Fertility trait	Heritability
USA	Pregnancy rate	0.04
Francia	Conception rate	0.02
Svizzera	Non return rate at 56d	0.01
	Calving to 1st insemination	0.04
Norvegia	Non return rate at 56d	0.01
Olanda	Non return rate at 56d	0.02
	Calving to 1st insemination	0.06
Israele	Conception rate	0.03
Irlanda	Calving interval	0.04
Finalandia	Days open	0.04
Danimarca	Non return rate at 56d	0.01
	Calving to last insemination	0.02
Germania-Austria	Non return rate at 90d	0.02
Svezia	N. of inseminations	0.03
	Calving to 1st insemination	0.04
Inghilterra	Calving interval	0.05
	N. of insemiantions for conception	0.03
	Non return rate at 56d	0.02

The implications of low heritability is that we need to collect fertility data on a large population of animals to achieve high reliability of genetic proofs, compared to higher heritability traits such as milk production. In general, as an example, with 200 daughters, a dairy bull has an expected reliability for calving interval of almost 80%; 80% reliability for milk yield is achievable, on average, with just 30 daughters for milk production.

In *Table 3*, are shown genetic and phenotypic correlations and heritabilities for yield traits, days open and productive life (*VanRaden et al*., 2004). Yield traits had higher heritabilities than fertility traits and showed an antagonist correlation among them.

Table 3

Genetic parameters (heritabilities on diagonal, genetic correlations above diagonal, and phenotypic correlations below diagonal) for first-lactation traits and productive life of Holstein (*VanRaden et al.*, 2004)

	Days open	Productive life	Milk	Fat	Protein	SCS
Days open	0.037	–0.59	0.38	0.33	0.32	0.30
Productive life	–0.20	0.076	0.03	0.04	0.06	–0.31
Milk	0.11	0.13	0.264	0.44	0.81	0.25
Fat	0.09	0.11	0.69	0.226	0.58	0.14
Protein	0.10	0.14	0.90	0.75	0.224	0.26
SCS	0.05	–0.13	–0.09	–0.09	–0.06	0.108

Correlations, means, standard deviation and heritabilities for reproductive traits of Holstein are provided in *Table 4*. These results supports days to first breeding is an important component of fertility days to last breeding were more genetically correlates with days to first breeding (0.85) than with number of inseminations (0.61) or nonreturn rate (-0.21). At the contrary, gestation length contributes very little to the variance of calving interval. Therefore, for genetic evaluation, traits as days to first and last breeding, nonreturn rate seem to be more promising for predicting the fertility genetic index.

Table 4

Genetic parameters (heritabilities on diagonal, genetic correlations above diagonal, and phenotypic correlations below diagonal), means, and SD for Holstein reproductive traits

	Genetic parameters					
Reproductive traits	Days to first breeding	Days to last breeding	Insemi nations	Nonreturn rate at 70 d	Gestation length	Mean ± SD
Days to first breeding	0.066	0.85	0.15	0.24	–0.01	90 ± 35
Days to last breeding	0.41	0.040	0.61	–0.21	–0.01	141 ± 75
Inseminations, no.	0.00	0.76	0.018	–0.88	0.02	2.1 ± 1.3
Nonreturn rate at 70 d	0.00	–0.32	–0.57	0.010	–0.03	0.55 ± 0.48
Gestation length, d	0.00	–0.02	–0.02	0.01	0.103	279 ± 5

GENETIC EVALUATION

Accurate genetic evaluations for fertility requires exploitable genetic variation to exist. More importantly routine access to accurate data on sufficient numbers of animals to generate accurate estimates of genetic merit is required. Due to the known genetic antagonism between milk yield and fertility (*Berry et al.*, 2011) some of the genetic

evalutations include milk yield as a predictor of fertility. However, the new fertility traits generated and recorded in many countries, such as the number of days from calving to first service, pregnancy rates during particular periods of the breeding season and calving rates within a pre-defined period of the calving season, body condition score, as indirect measurement, were very useful to improve estimated breeding values for fertility. Due to this worldwide situation, the fertility traits is included in the overall breeding indexes in many countries and their relative weight, as percentage of total merit indexes, ranges from 0 to 18,5% (*Minery et al*., 2008; *Canavesi*, 2009). In *Table* 5, are reported the relative emphasis on fertility and traits as percentage of total merit indexes of the most important countries in Holstein Friesian that are involved in the international genetic evaluation and in the worldwide semen market. *Minery et al*. (2008) showed that in comparing with previous years, there is a general increase of weight on fertility in the recent years, associated with a decrease of emphasis on the production traits.

Table 5

Relative emphasis (%) on fertility and other production, type and functional traits as percentage of total merit index (*Minery et* al., 2008; *Canavesi,* 2009

Country	% of Total Merit Index						
	Yield	**Type**	**Longevity**	**Somatic Cells Count**	**Fertility**	**Calving**	**Others**
United States (TPI)	45	29	10	5	8	3	-
Germany (RZG)	45	15	20	7	10	3	-
Netherlands (NVI)	40	27	8	9	16	-	-
France (ISU)	50	12,5	12,5	12,5	12,5	-	-
Canada (LPI)	51	27,2	6,8	5	10	-	-
Italy (PFT)	49	23	8	10	10	-	-
DFS* (S-Index)	34	16	6	14	9	6	15
New Zealand (BW)	61	18	5	7	9	-	-
Great Britain (PLI)	45,2	9,7	21,1	5,5	18,5	-	-

*Denmark, Finland, Sweden

The variety of traits considered in national fertility evaluation is continuously increasing. In recent years, fertility has regularly increased in total merit indexes in all countries and this trend can be considered a positive aspect to improve the fertility performances of dairy cows; at the contrary, the risk of using similar total merit indexes among countries is to increase the inbreeding. Inbreeding results from the mating of related individuals and it is also increasing within highly selected cattle population. In US, Holstein breed has rate of inbreeding of 0.2% per year (*Thompson et al*., 2000) correpsonding to an "effective" population size (*Ne*) of 50. Low *Ne* causes inbreeding and loss of genetic variation in a population. The current *Ne* of 50 in the US Holstein is lower than required to maintain genetic diversity in a population, but the decrease in *Ne* of Holstein and other dairy breeds is a recent phenomenon so little genetic variance has been lost to date.

However, inbreeding is not currently a serious problem, but if it continues to rise it will become a real problem in the next future. Inbreeding has three major undesirable effects. It causes inbreeding depression, including an increase in the incidence of abonmalities caused by recessive alleles, loss of genetic variance an random drift in the

population means. Inbreeding depression reduces the value of many traits, articularly those related to fitness, such as fertility, ability to remain healthy, and other traits indirectly affecting welfare.

CROSSBREEDING STRATEGY

As alternative strategy at selection for pure breed and, at risk of inbreeding, is the crossbreeding. The crossbreeding has gained considerable acceptance and uptake on the strength of sound scientific results. Fundamentally a successful crossbreeding strategy aims to introduce favourable genes from another breed (breeding programme) that has been selected more strongly for traits of interest, to remove the negative effects associated with inbreeding depression, and to capitalise on heterosis or hybrid vigour. Several research studies were conducted to evaluate the effect of crossbreeding strategy on fertility performances. Much of the benefit is attributed to substantial improvements in cow fertility, indicating that crossbreeding can provide a "quick fix" solution to many of the repercussions of past selection on milk production alone.

Prendiville (2009) showed large differences in fertility performance between Jersey×Holstein-Friesian crossbred cows compared with both groups of purebred cows (Holstein-Friesian and Jersey). Averaged over the first five years, the pregnancy rate to first service of the Holstein-Friesian was 47 per cent, but the Jersey×Holstein-Friesian crossbred was markedly superior at 62 per cent. The six week in-calf rate was 56 per cent for the Holstein-Friesian and 70 per cent for the Jersey×Holstein-Friesian crossbreds. The 13 week in-calf rate of 90 per cent for the Jersey×Holstein-Friesian crossbreds was eight percentage units superior to the Holstein-Friesian. The fertility performance of the purebred Jersey was no better than that of the Holstein-Friesian. This leads to the conclusion that the superior performance of the Jersey crossbred cows is largely attributable to hybrid vigour. Again, productivity was not compromised with the crossbred cows compared to the Holstein-Friesian cows.

An economic analysis conducted in 2009 (base milk price of 27 c/l, and cull and calf values reflective of that time; Prendiville, 2009) estimated superior profit (per lactation) for the Norwegian Red×Holstein-Friesian and Jersey×Holstein-Friesian cows of +€130, and +€180, respectively, compared to the pure Holstein-Friesian cows. This equates to almost €13,000 and €18,000 more profit annually in a 100 cow herd for Norwegian Red crossbreds and Jersey crossbreds, respectively.

Heterosis, or hybrid vigour, is a form of non-additive genetic variation that is not 'passed on' through generations. Heterosis, however, is maintained to varying degrees in advanced generations of crossbreeding. As far as a long term strategy is concerned, three options exist. These are as follows:

I. Two-way crossbreeding. This entails mating the F1 cow to a sire of one of the parent breeds used initially. In the short term, heterosis will be reduced but over time averages 66.6 per cent.

II. Three way crossing. Simply use a high estimated breeding index (EBI) sire of a third breed. When the F1 cow is mated to a sire of a third breed, hybrid vigour is maintained at close to 100 per cent. Then revert back to using high EBI Holstein-Friesian sires. With the reintroduction of sires from the same three breeds again in subsequent generations the heterosis levels out at 85.7 per cent.

III. Synthetic crossing. This involves the use of F1 or crossbred bulls. In the long term a new (synthetic) breed is produced. Heterosis in this strategy is reduced to 50 per cent initially and is reduced gradually with time.

The results presented strongly suggest that both Jersey×Holstein-Friesian and Norwegian Red×Holstein-Friesian can play a fundamental role as a part of a crossbreeding strategy to increase health and fertility without compromising production on dairy farms. For selection among breeds to be useful, an accurate across-breed genetic evaluation is vital and of large interesting in the future.

FUTURE RESEARCH IN GENETICS OF FERTILITY

Interaction of genetics and environment aspects (GxE) is an important field of the future research, as animals tend to adapt to the environment they are selected in, it is likely that selection for increased yield may also lead to environmental sensitivity. Harris and *Winkelman* (2000) and *Verkerk et al*. (2000) reported significant differences between cows of New Zealand origin and those of North American origin for conception rate, services per conception, and days to first service. These studies indicate that the negative genetic correlations between production, fertility and health in modern dairy cows, already large when producing in an intensive production enviroment, are even larger when cows are producing in a less intensive production environment. Therefore, the increase in negative genetic correlation between production and fitness traits in less favourable environments is indicative of a decline in adaptability associated with selection for increased yield in the modern dairy cows.

Another, important field of the present and future research is the genomic selection, already available in many highly selected dairy cattle populations. Simulation studies (*Veerkmap and Beerda*, 2007) have shown that genomic selection improves the accuracy of selecting juvenile animals compared with traditional breeding methods and compared with selection using information from a few genes or QTL only. Research in the areas genomics and proteomics promise to make genetic selection even more effective. The genomic and proteomics technologies combined with the bioinformatics tools that support the interpretation of gene functioning and protein expression facilitate an exciting starting point for the development of new management strategies and tools for the improvement of reproductive performance. Another promising research area is the expanding genomic selection to alternative breed sires and genotyping of crossbred cows producing in many environments. Required, however, is a very large database of animals; the larger the database the greater the improvement in accuracy from genomic selection.

Access to genomic information on individual animals can also be useful in predicting crossbred performance resulting from a given mating or identifying mates that are complementary. Calving interval is an accumulation of different individual fertility traits including the duration from calving to first ovulation, the intensity and duration of oestrus expression, the ability to conceive and maintain pregnancy to first service, and gestation length. Faster genetic gain will be achievable if selection were to be undertaken on improving all of the individual traits individually. Also, minimising the influence of management and recording errors (i.e., improved ability of individual farmers to detect oestrus, better record keeping, etc.) can also increase the heritability and therefore increase genetic gain, assuming routine access to the new traits is also available to identify the genetically elite animals.

CONCLUSIONS

Fertility in dairy cows during the last decades is globally decreasing with increasing levels of production. Future strategies to improve dairy cow fertility are needed for the benefit of the dairy industry and for cow welfare and should be based upon an integrative approach of these events.

Selection for high production reduces fertility and the reproductive traits have shown to be less heritable and more variable than production or type traits. However, fertility is partly controlled by animal genetics and this is well known and proven, hence animal fertility can be improved through genetics. The tools, as total merit indexes and selected bulls are available in many countries to identify genetically elite animals for fertility, without compromising other performance traits.

Across-breed genetic evaluations seem to be an interesting opportunity to select the genetically elite animals, irrespective of breed. Breed complementarity and heterosis, obtainable through crossbreeding, can provide an additional gain in performance, particularly in relation to fertility. An optimal breeding program should form an integral part of a strategy at individual herd and international level to increase farm profit through improving herd fertility without compromising other performance traits. An important field of the present and future research is the genomic selection, already available in many highly selected dairy cattle populations. A promising research areas are the studies on the GxE interaction and the genomic selection to alternative breed sires and genotyping of crossbred cows producing in many environments.

In conclusion, if infertility is a major cause of elimination of the cows and the very high cost, the strong selective pressure for the production of milk around the world has led to a sharp decline in reproductive efficiency in the breeding of dairy cows. However, from the genetic point of view the fertility is more variable of the type and the production, and the selection is possible, while expecting a genetic progress slow. Several countries are selecting for fertility, either by using direct measurements (eg pregnancy rate) and indirectly (eg BCS) and farmers have at their disposal bulls evaluated for these traits. New research frontiers, as the genomic selection and proteomic analyses could help the breeder, which must still continue to record all inseminations and, in general, all the reproductive events. The timely and proper recording of data in fertility leads to archives to analyze high-quality and you can have the most reliable breeding values, maximizing the genetic progress.

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Microsatellite analysis of population structure in Slovak Pinzgau cattle

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ABSTRACT

The aim of the study was improve knowledge about population structure of Slovak Pinzgau cattle using genetic markers. Observed population structure was characterized by use of eight microsatellites. Each locus was tested for deviations from the Hardy-Weinberg Equilibrium (HWE). In general, breed was in genetic equilibrium, only locus BM1824 deviated from HWE. Cluster algorithms identify groups of related individuals without reference to prior information of the genetic subdivision. We considered 3 clusters that capture the major structure of the data (representative K value) and most likely reflect genealogical structuring. The chosen set of microsatellites confirmed the suitability for genetic structure assessment and its usefulness in determination of the subpopulations for Pinzgau cattle in Slovakia.
(Keywords: genetic structure, microsatellites, Pinzgau cattle, subpopulations)

INTRODUCTION

Many industrial breeds currently suffer from inbreeding, and genetic resources in cattle, sheep, and goats are highly endangered, particularly in developed countries (*Taberlet et al.*, 2008). Genetic diversity within farm animal species refers to the extent of genetic variation within and among breeds, strains and lines in order to preserve the highest intraspecific variability (*Lenstra et al.*, 2012). Maintaining genetic variation is an important requirement for future animal breeding strategies, to match animals to a variety of husbandry systems and for adaptation to environmental changes. In addition, genetic diversity of livestock species is of considerable scientific interest for understanding phenotypic variation (*FAO*, 2007) and for reconstructing the history of livestock (*Ajmone-Marsan et al.*, 2010; *Groeneveld et al.*, 2010).

Slovak Pinzgau cattle are divided into two separate populations. The first is represented by dual-purpose type (dairy) and the second by beef suckler cows (beef). Pinzgau cattle are an original Alpine breed, which had been imported to Slovakia approximately 200 years ago. Thanks to its unique traits as longevity, fertility, health, grazing ability it had been bred in mountain regions of northern Slovakia, but there is significant decline of the population in recent years. Due to this, the population can be considered endangered and it is necessary to assess genetic variability. Taking in the account the situation alternatively breeding programs were optimised (*Kadlečík et al.*, 2004), development were monitored (*Kasarda et al.*, 2008) and analyses of genetic diversity were performed (*Pavlík et al.*, 2013).

Microsatellite markers have been widely used for population genetic analyses and structure of livestock species, as they are informative and can successfully elucidate the relationships between individuals and populations, including also cattle populations (*Sun et al.*, 2007). Microsatellites have been commonly used to assess within-breed genetic diversity and inbreeding levels, introgression from other species, genetic differentiation, admixture among breeds (*Ginja et al.*, 2009) and to define conservation priorities (*Lenstra et al.*, 2012).

Pritchard et al. (2000) described a Markov chain Monte Carlo (MCMC) scheme clustering individuals into populations and estimating the probability of membership (or, for the admixture ,odel, the proportion of membership) in each population.

The most widely used measures of population structure are Wright's F statistics (*Wright*, 1931), which partition the genetic variation in a within-subpopulation component (average subpopulation inbreeding coefficient F_{IS}) and between-subpopulations component (fixation index F_{ST}), with the inbreeding in the total population described by the inbreeding coefficient F_{IT} (*Lenstra et al.*, 2012). In case of heterozygosity decreasing in population F_{IS} value will be positive and opposite, if there is a sufficient number of heterozygotes, this value will be negative (*Hamilton*, 2009). F_{ST} measure provide important insight into the evolutionary processes that influence the structure of genetic variation within and among populations, and they are among the most widely used descriptive statistics in population and evolutionary genetics (*Holsinger and Weir*, 2009). To calculate these indices, one needs first to define groups of individuals and then to use their genotypes to compute variance in allele frequencies. Thus, a fundamental prerequisite of any inference on the genetic structure of populations is the definition of populations themselves. Population determination is usually based upon geographical origin of samples or phenotypes. However, the genetic structure of populations is not always reflected in the geographical proximity of individuals. Populations that are not discretely distributed can nevertheless be genetically structured, due to unidentified barriers to gene flow. In addition, groups of individuals with different geographical locations, behavioural patterns or phenotypes are not necessarily genetically differentiated (*Evanno et al.*, 2005). Bayesian approach uses a Monte Carlo-Markov Chain (MCMC) simulation to infer the most probable number of population clusters and to estimate the proportional contribution of each of the assumed subpopulations to the genotypes of an individual (*Pritchard et al*., 2000).

The aim of this study was to assess genetic structure of Slovak Pinzgau cattle population based on polymorphism at microsatellite loci using statistical programs. This should allow improve our knowledge of population structure and genetic variability with using for preservation of the breed in the original phenotype supported by the current selection schemes and breeding programmes.

MATERIAL AND METHODS

Random selected 302 cows of Pinzgau cattle from four Slovak farms were analysed. Both farming types were represented (beef and dual-purpose), purebred and crossbred animals. DNA was isolated from hair roots and amplified in one multiplex PCR with 8 microsatellites (TGLA122, CSSM66, TGLA227, ILST006, CSRM60, ETH3, BM1824, SPS115). To determine the polymorphism of microsatellite DNA sequences was used fluorescent fragmentation analysis by ABI PRISM 310 Genetic Analyser and the allele sizes were evaluated. Microsatellite analysis using fluorescently-labelled primers and capillary fractionation is the pre-eminent method for the genetic analysis of eukaryotic

organisms. All loci were tested for deviations from the Hardy-Weinberg equilibrium (HWE) using a permutation version of the exact test given by *Guo and Thompson* (1992) provided in PowerMarker V3.25 software (*Liu and Muse*, 2005).

First, observed animals were divided into subpopulations based on farm, where are the animals living, breed type, respectively level of admixture of other breeds, year of the birth and line of father. To describe the properties of a subdivided population F-statistics, genetic identity and distance measures were estimated using above-mentioned software. F_{IS} and F_{ST} values per locus with standard deviation (SD) estimated on 1000 bootstrap replicates were computed. A priori divisions were tested using GENETIX 4.05.2 (*Belkhir et al*., 1996-2004), F_{ST} significance and corresponding analyses showing admixed population was observed.

Second, the Bayesian clustering algorithm implemented by the STRUCTURE 2.1 software (*Pritchard et al*., 2000) was used to infer the population structure. The program enables estimation of a 'hidden structure', that is the number of different clusters (K partitions) obtained without using any a priori information about individual membership (population and/or breed). Furthermore, the program is able to determine the corresponding fraction of an individual's genome derived from an ancestry in one of the clusters (K) determined by the program. The program STRUCTURE uses the MCMC method, see also *Falush et al*. (2003), and estimates the natural logarithm of the probability (Pr) of the observed genotypic array (G), given a preassigned number of clusters (parameter K) in the dataset [ln Pr(G|K)]. In a Bayesian set-up the estimate of ln Pr(G|K) is a direct indicator of the posterior probability of having K number of clusters, given the observed genotypic array (G). To obtain a representative value of K for modelling the data, we ran 10 independent runs of the Gibbs sampler for each K between 1 and 8 with a burn-in length of 10^5 followed by 10^5 iterations. In all runs we used default settings, that is, an admixture model with correlated frequencies and the parameter of individual admixture alpha set to be the same for all clusters and with a uniform prior. After determining the most likely number of subpopulations, the contribution of each K to whole population was estimated.

RESULTS AND DISCUSSION

Out of the 8 analysed loci only BM1824 showed highly significant ($P \leq 0.001$) HWE deviations across breed. The overall average of fixation index was close to zero ($F_{IS} = -0.0039$) which means the reduction of heterozygosity in the whole population was not observed. The F_{ST} has reached following values according to the division method: 0.0188 by farm, 0.003 by breed type, 0.053 by year of the birth and 0.0669 by paternal lines. Detection of possible subpopulation structures provided us with initial view at the genetic structure of Slovak Pinzgau cattle. Positive F_{ST} values indicate a deficiency in heterozygotes in the subpopulations, whereas in the whole population appears to be sufficient heterozygosity, what may imply the Wahlund effect. Generally, F_{ST} values between 0.05 and 0.3 are typical for differentiation of livestock breeds, with a value over 0.15 indicating significant differentiation (*Frankham et al.*, 2002), although much smaller values can be significant (*Lenstra et al.*, 2012). A priori divisions were tested using GENETIX and no statistical significance was observed as well as correspondence analyses showed rather admixed population in all cases.

We applied STRUCTURE to measure the population structure as the implemented algorithm uncovers 'hidden structure' without using any a priori knowledge about the number of clusters present in dataset. In order to illustrate a decision on the most likely

number of clusters present in the dataset (the most likely parameter K), in *Figure 1*, we presented ln Pr(G|K) values for all STRUCTURE runs. Over the entire cattle population, ln Pr(G|K) increased from K=1 to K=3, after which it began to decline. It was assumed that the most likely K is that where ln Pr(G|K) is maximised. We therefore considered K=3 as being the number of clusters that capture the major structure of the data (representative K value). The difference of ln Pr(G|K) between K=1, K=2 and K=3 are small (less than 200 between K=3 and K=2) so the structure obtained is relatively weak and most likely reflecting genealogical structuring. A quantification of how likely each individual is to belong to each group is given in *Figure 2*.

Figure 1

Ln Pr(G|K) values presented as a function of the number of clusters. The largest ln Pr(G|K) values within each K (among 10 runs) are presented with cirkles

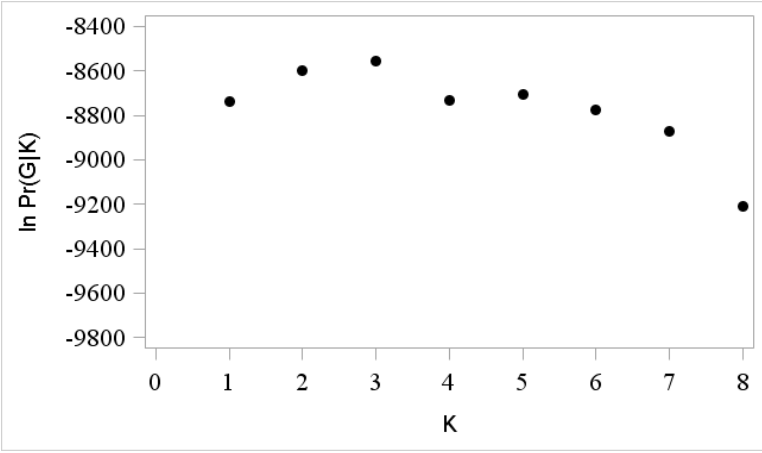
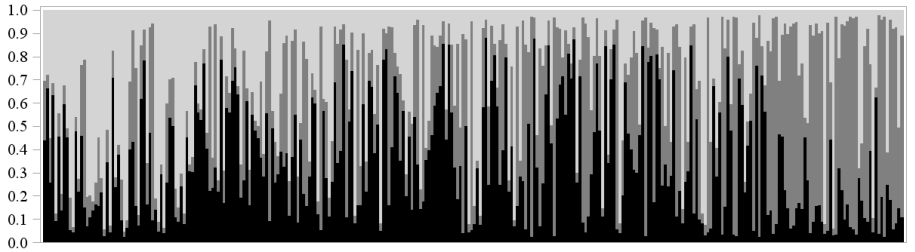


Figure 2

Graphical presentations of the population structure analyses for a sample of 302 Pinzgau cows (without a priori information about subpopulations). Each cow is represented by a single vertical line broken into K colour segments, with lengths proportional to the estimated membership of the inferred cluster



CONCLUSIONS

Genetic structure of Pinzgau cattle population has been analysed using set of 8 microsatellites. The Bayesian approach implemented by the STRUCTURE software was effective in detecting number of clusters. The mean value of ln Pr(G|K) increased up to K=3 and dropped afterwards, indicating the most likely value to be K=3. No of a priori subdivision was significant, however we assumed that population division is based on genealogical information. Concrete character of population structure is a subject of further investigation. The used set of microsatellites can be applied in more detailed studies in the future by analysing more breeds, larger numbers of animals per breed. This should allow improve our knowledge of origin and phylogenetic relationships to other breeds and provide a basis for preservation of the breed in the original phenotype favoured by the current selection schemes and breeding programmes

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Estimation of inbreeding and effective population size in Istrian cattle using molecular information

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ABSTRACT

To provide preliminary insight in the conservation risk status in Istrian cattle we analysed ROH inbreeding and effective population size in 15 individuals, mostly bulls, using BovineSNP50K BeadChip. We obtained very high inbreeding level, although with broad confidence interval, and very low effective population size. While the results obtained are preliminary (small sample size) and should be treated with caution, the high recent inbreeding and small effective population size suggest additional monitoring of the conservation risk status of the Istrian cattle.
(Keywords: Istrian cattle, Inbreeding, Runs of homozygosity, effective population size, single nucleotide polymorphism)

INTRODUCTION

Istrian cattle, colloquially called Boškarin, is the autochthonous breed spread mainly over the Istrian peninsula. The breed belongs to the group of grey cattle breeds that are scattered over the Balkan and neighbouring countries (Croatia, Bulgaria, Greece, Hungary, Italy, Romania, Serbia, Turkey and Ukraine) and that are considered as direct descendants from the Auroch (Bos primigenius). In the last 50 years the number of Istrian cattle individuals has been reduced dramatically. Inbreeding level and effective population size (*Ne*) are among the most important conservation genetic parameters. Classical inbreeding and *Ne* estimates rarely work well in real populations as they are mostly based on inaccurate pedigree records or, in case of *Ne* estimation, on robust demographic parameters that do not completely recognise the history of the population (bottlenecks, preferential mating or population subdivision). The rapid development of new molecular technologies enabled high-throughput genotyping of individual animals at available prices. Consequently, those technological achievements provide new views on old problems and reinforce estimation of inbreeding and *Ne* from molecular markers. Runs of homozygosity (ROH) were recently proposed as a useful concept in quantifying individual inbreeding in humans (*McQuillan et al.,* 2008), cattle (*Ferenčaković et al.,* 2011; *Purfield et al.,* 2012) and pigs (*Bosse et al.,* 2012), performing even better than traditional estimates calculated from the pedigree. *Sved* (1971) and *Hill* (1981) showed that linkage disequilibrium (*LD*) could be used to estimate *Ne*. While theoretical basis has been established before, the practical use of *LD* in estimating *Ne* started by *Hayes et al.*, (2003) and, further, continued by *Tenesa et al.,* (2007); *Qanbari et al.,* (2009).

The aim of this study was, based on high-throughput genotypes (BovineSNP50K BeadChip), to estimate inbreeding level and effective population size in Istrian cattle. The results obtained will contribute to the conservation management strategy of the Istrian cattle.

MATERIAL AND METHODS

Samples (15) representing Istrian cattle population, mostly bulls, were either taken from the blood (randomly chosen from several private farms in Istria, or were obtained as semen straws (three bulls) from CRSH d.o.o. in Krizevci (www.crsh.hr). As the number of Istran bulls is extremely small we have considered our sample as representative, although, we are aware that larger sample would be more adequate.

After ROH calculation quality control that was performed according to *Ferenčaković et al.* (2013b) we proceed with analyses including information from 42265 SNPs (%), placed on 29 autosomes and with average distance of 59 kb between adjacent SNPs. ROH segments were identified as a part of the genome in which 15 or more consecutive homozygous SNPs at a density of one SNP on every 100 kb are not more than one Mb apart. ROH calculations were done by SNP & Variation Suite (v7.6.8 Win 64; Golden Helix, Bozeman, MT, USA www.goldenhelix.com). The general formula for calculating F_{ROH} from chip data is $F_{ROH}= L_{ROH}/L_{AUTOSOME}$, where L_{ROH} is the total length of all ROH in the genome of an individual while $L_{AUTOSOME}$ refers to the specified length of the autosomal genome covered by SNPs on the chip (here 2,543,177 kb). For each bull, we calculated three inbreeding coefficients ($F_{ROH>4Mb}$, $F_{ROH>8Mb}$ and $F_{ROH>16Mb}$) based on ROH of different minimum lengths (>4, >8 or >16). Different ROH inbreeding coefficients are expected to have differently remote common ancestors (for details see *Curik et al*., 2014).

Effective population size (Ne) was estimated following the approach described in *Flury et al*. (2010) respecting functional relationship of *Ne* with correlation r^2 and recombination rate (*c*), here inter-marker genetic distance between two considered loci with assumption that 1 Mb = 1 cM. Two slightly different formulas were used, one described in Sved (1971) where $r^2=1/(1+4 \cdot c \cdot Ne_1)$ and the other described in Weir and Hill (1980) where $r^2=1/(1+4 \cdot c \cdot Ne_2)+(1/n)$ with $n=2 \cdot$number of animals (bulls) used in the calculation as a correction factor for a sample size induced LD. Only SNPs with adjacent r^2 values from 0.01 to 0.99 were used in the calculation by *Uimari and Tapio* (2011). Finally, time defined effective population size Ne_T was derived from 40 marker distance derived categories as described in *Flury et al.* (2010). Current effective population size was predicted based on the regression analysis of estimated values in previous 150 generations. LD (r^2) was estimated using SNP & Variation Suite (v7.6.8 Win 64, Golden Helix, Bozeman, MT, USA www.goldenhelix.com). Data manipulations, numerical calculations and graphical visualisations were done by procedures included in SAS 9.3 (*SAS Institute*, 2011).

RESULTS AND DISCUSSION

Summary statistics of the ROH estimated inbreeding level ($F_{ROH>4Mb}$, $F_{ROH>8Mb}$ and $F_{ROH>16Mb}$) in 15 Istrian cattle bulls are presented in *Table 1*. The estimates obtained (mean and standard deviations) were much higher than those obtained in Brown Swiss, Fleckvieh, Norwegian Red and Tyrol Grey by *Ferenčaković et al.* (2013a) or in Pinzgauer by *Ferenčaković et al.* (2013b). However, one should be aware that the

confidence limits are very broad with values comparable to any population studied so far. One individual had extremely high close inbreeding ($F_{ROH>8Mb}$=0.351 and $F_{ROH>16Mb}$=0.287) indicating the absence of mating strategy respecting avoidance of close inbreeding.

Table 1

Summary statistics of inbreeding calculated from ROH with different lengths (>4 Mb, >8 MB and >16 MB) based on Illumina BovineSNP50K BeadChip in 15 Istrian cattle bulls

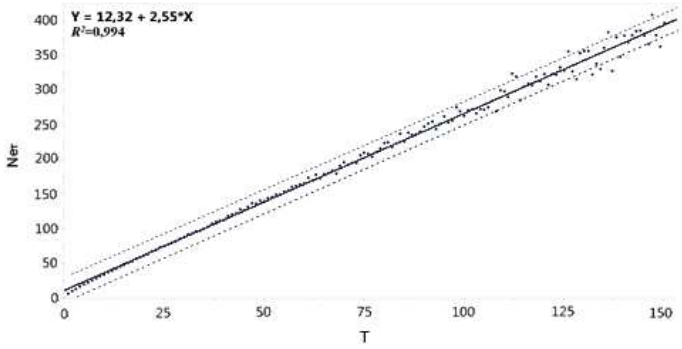
Inbreeding coefficient	Mean	Lower 95% CI	Upper 95% CI	Standard deviation	Range
$F_{ROH>4Mb}$	0.093	0.039	0.147	0.092	0.002-0.368
$F_{ROH>8Mb}$	0.081	0.029	0.133	0.091	0.000-0.351
$F_{ROH>16Mb}$	0.075	0.014	0.096	0.078	0.000-0.287

CI = Confidence interval

Historical estimates of the effective population size (Ne_T) during last 150 generations showed rather linear decrease of 2.55 individuals per generation while predicted current generation effective population size (Ne_0) was equal to 12.32 with 95% confidence interval ranging from 9.58 to 15.06 individuals (*Figure 1*).

Figure 1

Linear regression with 95% confidence interval presenting relationship between historical effective population size (Ne_T) and number of generations in the past (T) while Ne_T values were previously estimated from genomic data of 15 Istrian cattle individuals



Thus, the linear regression function was Ne_T=12.32+2.55·T with extremely high coefficient of determination (R^2=0.994). The obtained prediction for the current effective population size of Istrian cattle was surprisingly small. According to the Croatian Agricultural Agency report (2013) the breed status is highly endangered with Ne estimated to 151.59 (721 cows and 40 bulls) when calculated from the sex ratio

[Ne = (4·Nm·Nf) / (Nm+Nf), where Nm and Nf represent the number of breeding males and females, respectively].

Although, the sample size was very small, historical estimates of effective population size do represent large number of chromosomal segments originating from much larger number of individuals and, thus, should be less sensitive to the sample size. Still, the interpretation of the results should be considered with caution as we are not fully aware of the magnitude of potential bias resulting from one individual being highly inbred.

CONCLUSIONS

Although, the results obtained are preliminary (small sample size) and should be treated with caution, the appearance of high recent inbreeding in some individuals and small effective population size require additional monitoring of the conservation risk of Istrian cattle population.

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Polymorphism and allelic frequency of CSN1S1 in different cattle breeds in Bulgaria

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ABSTRACT

The present study aims at determining the *genetic polymorphism at the milk protein* genes *CSN1S1 and their allelic frequency in widespread and endangered cattle breeds raised in Bulgaria. Analyzed were 390 tissue samples of two widespread cattle breeds, i.e. 129 of the Bulgarian Brown Cattle and 129 of the Bulgarian Black and White Cattle, as well as of two endangered cattle breeds- 23 of the Rhodopean Short - Horned Cattle and 109 of the Iskar Cattle. Determined are significant differences in the frequency of the heterozygous genotypes, i.e. higher frequency in the local breeds. The Iskar Cattle stands out with the highest percentage of the BC heterozygous genotype, i.e.- 66.972% compared to the other breeds included in our study.*
(Keywords: polymorphism, allelic frequency, milk proteins, indigenous cows)

INTRODUCTION

The polymorphism of the milk proteins found in milk enjoys continuous scientific interest. Established is its relation with the milk production /Bech et al., 1990/, milk composition (*Robitaille et al*., 2002) as well as the process parameters in the manufacture of various cheese types (*Hill et al*., 1997; *Ng-Kwai and Hang*, 1998; *Mclean et al*., 1987; *Schaar et al*., 1985; *Van den Berg et al*., 1992). The studies carried out in this connection are the basis giving faith and hope that it is possible to effectively use the genetic polymorphism of the milk proteins as a genetic marker in the genomic selection in dairy cattle breeding.

The use of the polymorphic loci and their use as microsatellites for mapping and selection based on markers (*Simianer et al*., 2003) have a growing importance for preserving the specific genetic diversity of the local and endangered cattle breeds.

Dalvit et al. (2009) study the genetic diversity in endangered Bulgarian cattle breeds. Study of the polymorphism at the milk proteins in the local breeds has not been carried out.

The present study aims at determining the genetic polymorphism at the milk protein genes CSN1S1 and their allelic frequency in widespread and endangered cattle breeds raised in Bulgaria.

MATERIALS AND METHODS

Facts

Analyzed were 390 tissue samples of two widespread cattle breeds-129 of the Bulgarian Brown Cattle and 129 of the Bulgarian Black and White Cattle as well as of two endangered cattle breeds, i.e. 23 of the Rhodopean Short - Horned Cattle and 109 of the Iskar Cattle. Tissue samples were taken by an innovative marking technology according to which special containers with drying agents were put aside upon marking of each animal, object of the study.

Laboratory Analysis

DNA was purified from ear tags using the Maxwell®16 Tissue DNA Purification Kit (Promega) according to manufacturer's instruction.Twenty nanograms of purified DNA were used for the amplification of portion of the CSN1S1 gene using primers CSN1S1-10-F 5'-TGC CTA TCC ATC TGG TGC CTG G-3' and CSN1S1-10-R 5'-GCT CCA CAT GTT CCT GAG TAA TGG-3' and standard PCR condition. 5 µl of PCR products were purified using ExoI and SAP (ThermoScientific) for 15 min at 37 °C followed by an inactivation step at 85 °C for 15 min. Allelic discrimination was performed using the Genome™Lab SNP-Primer Extension Kit (BeckmanCoulter) following manufacturer's instructions and interrogation primer CSN1S1_SBE: 5'-TAT TAA TC CAT TGG CTC TGA GAA CAG TG-3'. Electropherograms produced by CEQ8000 automatic capillary sequencer (BeckmanCoulter) were analyzed using Genetic Analysis Software v9.0 (BeckmanCoulter).The genetic polymorphism of the milk proteins has been determined using PCR-RFLP analysis in the Padova University Laboratory. For data analysis the software product Systat 13 was used**.**

RESULTS AND COMMENTS

Table 1 shows the results in the allelic frequencies of the CSN1S1 genes in widespread and endangered cattle breeds in Bulgaria. Two alleles-B and C were detected in CSN1S1 gene. As seen from the *Table* in all breeds included in our study, allele B features higher frequency than allele C.

Table 1

Allelic frequency of CSN1S1 in different cattle breeds in Bulgaria

Allelic frequency	B	C
Bulgarian Brown Cattle	0.814	0.186
Bulgarian Black and White Cattle	0.748	0.252
Rhodopean Short - Horned Cattle	0.565	0.435
Iskar Cattle	0.619	0.381

In the animals of the Bulgarian Brown Cattle breed, determined is the highest allelic frequency of the B allele, i.e. 0.814 and the lowest allelic frequency of the C allele, i.e. 0.186. The allelic frequencies determined by us for the Bulgarian Brown Cattle, i.e. for the B allele-0.748 and for theC allele-0.252, are similar to those determined for the a.m. breed. These similarities in the frequencies in two of the most widespread cattle breeds in our country most probably is attributable to the focused selection on particular

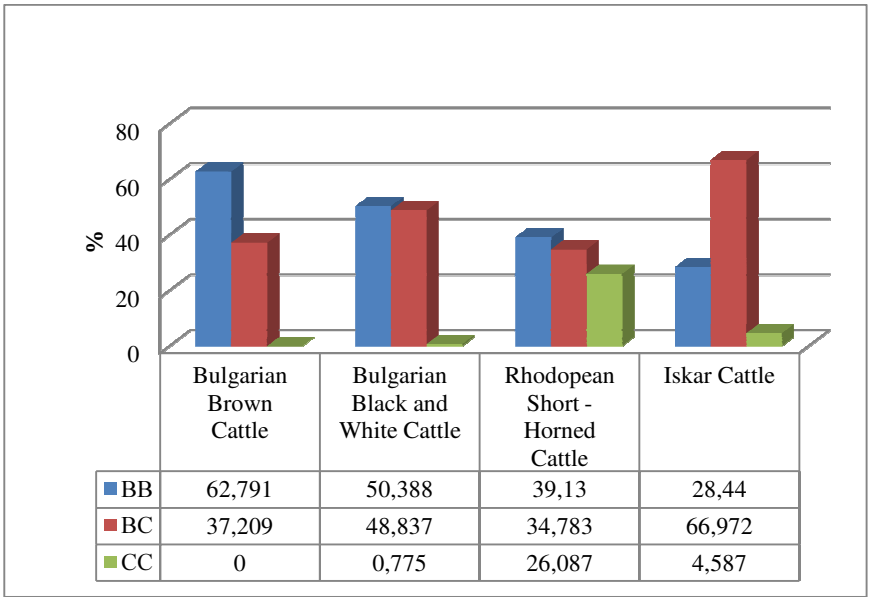
qualities. The results determined by us correspond to those published by *Smiltina et. al*., (2009), studying the population of the Latvian Brown Cattle. Other authors determine a higher frequency for the B allele, i.e. 0.86 and a lower frequency for the C allele, i.e. 0.14 in the Holstein cattle breed (*Yasemin et al*., 2006; *Zakizadeh et al*. 2013; *Micińsk et al*. 2008).

In the Rhodopean Short - Horned Cattle the lowest frequency of the B allele-0.565 and the highest frequency of the C allele-0.435 is determined. Similar values are also determined for the Bulgarian Rhodopean Cattle-the B allele being 0.503 and the C allele being 0.497 which most probably is attributable to the fact that this breed has been produced on the basis of the Rhodopean Short - Horned Cattle (*Yordanova et. al*., 2013). The Iskar Cattle features the following allelic frequencies: for the B allele – 0.619 and for the C allele – 0.381. According to *Boettcher et al*. (2004), the B allele is wider spread than the C allele.

Figure 1 shows the polymorphism of CSN1S1 in different cattle breeds raised in Bulgaria. *Caroli et al*. (2009) state that CSN1S1 features 9 genetic variants (A, B, C, D, E, F, G, H, I) as most common are the B and C alleles. The results determined by us correspond to those determined by the author.

Figure1

Polymorphism of CSN1S1 in different cattle breeds in Bulgaria



	Bulgarian Brown Cattle	Bulgarian Black and White Cattle	Rhodopean Short - Horned Cattle	Iskar Cattle
BB	62,791	50,388	39,13	28,44
BC	37,209	48,837	34,783	66,972
CC	0	0,775	26,087	4,587

The above figure shows that CSN1S1 features 3 genotypes, i.e. BB, BC and CC. Difference in the percentage of the afore-mentioned genotypes is significant and it may be claimed that there is a significant difference between the widespread and the endangered cattle breeds. The Bulgarian Brown Cattle stands out with the highest percentage in the BB homozygous genotype – 62.791% and the BC heterozygous genotype has a much lesser value, i.e. – 37.209% while for the CC homozygous

genotype the percentage is not determined. The results determined by us confirm the results published by *Smiltina et. al*. (2009) showing the non-existence of homozygous animals of the CC genotype in cows from the Latvian Brown Cattle. The frequencies of the homozygous BB and heterozygous BC genotype determined in the Bulgarian Black and White Cattle have similar values.

Determined are significant differences in the polymorphism of the Rhodopean Short - Horned Cattle and the Iskar Cattle. Determined are similar values of the percentage frequency of the genotypes of CSN1S1 in the Rhodopean Short - Horned Cattle as the results we determined are as follows: BB- 39.13%, BC- 34.783% and CC- 26.087%. The Iskar Cattle features the highest percentage of the heterozygous genotype, i.e. BC- 66.972%, while the homozygous genotypes BB and CC have lower values, i.e. 28,44% and CC- 4,587% respectively. *Zakizadeh et al*. (2013) determine similar results in studying the genetic frequency of CSN1S1 in Holstein and indigenous breeds in Iran.

COMMENTS

The differences in the B and C allele frequencies in widespread and endangered cattle breeds in Bulgaria most probably result from the various selection objectives and approaches-intensified import of genetic plasma in the widespread breeds and natural breeding in the case of the Iskar Cattle. We need to mention also the limitations resulting from the population number. In the Rhodopean Short - Horned Cattle there are also specific factors such as prevailing artificial insemination in the breeding area resulting from the limited genetic diversity offered by the male cattle in the population. This might be the reason for the damaged genetic structure and balance in this breed determined by *Dalvit et al*. (2009).

CONCLUSIONS

1. Two alleles-B and C were detected in the CSN1S1 gene as the B allele features a higher frequency than the C allele in the widespread and endangered local breeds. Determined are significant differences in the frequency of the two alleles in the breeds studied by us.
2. Determined are significant differences in the frequency of the heterozygous genotypes as their frequency in the local breeds is higher. The Iskar Cattle features the highest percentage of the BC heterozygous genetic type - 66.972%, compared to the other breeds included in our study.

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A stochastic simulation study for the comparison of different methods to calculate a total merit index

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ABSTRACT

In order to compare four different methods for calculation of a total merit index, a stochastic simulation study was conducted. Five normally distributed traits were chosen to represent the blocks dairy, beef and fitness of a simulated cattle population. The reference method was a full multivariate evaluation based on raw data. The other three methods were based on selection index theory with different approaches to calculate covariances between estimated breeding values. Additionally a focus was put on the implications of varying the residual covariances between traits. All selection index methods showed similar results. However, the method currently used in the joint genetic evaluation led to noticeable biases in EBVs especially when residual covariances between traits were high. Residual covariances seem to have an important impact when calculating a total merit index and should not be ignored. Results of the present study encourage to move towards a multitrait approach or at least to account for residual covariances when combining EBVs into a total merit index.
(Keywords: total merit index, multitrait evaluation, stochastic simulation)

INTRODUCTION

The total merit index (TMI), which is a function of different estimated breeding values (EBV), is used as one of the most important selction criterions worldwide (*Miglior et al., 2005*). In modern dairy cattle breeding programs, the TMI is commonly based on different production and increasingly on functional traits. Typically EBVs of different traits are weighted, concerning their economic importance and combined to a TMI (*Hazel and Lush*, 1942). The use of selection index theory is however faced with some challenges: Traits or group of traits are usually evaluated separately based on different statistical models, and hence true genetic or/and phenotypic correlations or heterogeneous reliabilities are neglected (*Ducrocq et al.*, 2001). This is also the case in the joint genetic evaluation of Austrian and German dairy cattle breeds. The TMI and several sub-indices for all cattle breeds except Holstein is based on a selection index method (*Hazel and Lush*, 1942) which was proposed by *Miesenberger* (1997). The TMI of Fleckvieh (dual purpose Simmental) and Brown Swiss currently consists of more than 20 different production and functional traits. EBVs for the TMI as well as for several sub-indices are estimated either univariately or multivariately in different linear or non linear models. Subsequently EBVs are combined to TMIs or to other sub-indices assuming that residual covariances between traits or group of traits are zero. A full

multivariate estimation of all traits based on raw data could be considered as the optimum methodology but is usually not feasible (*Mrode*, 2014). Although computer power and capacity is increasing quickly it is still demanding to compute all traits, which are included in a TMI, together. Experiences of the last years suggest that particularly TMIs with low reliabilities (r^2) are slightly overestimated. Much effort is put on an approximate two-step procedure (*Fuerst et al.,* 2014; *Pfeiffer et al.,* 2014), which was proposed by *Ducrocq et al.* (2001) and validated by *Lassen* et al. (2007). However, alternative combinations of independently estimated breeding values are also evaluated. Apart from the method proposed by *Miesenberger* (1997), two additional similar methods described by *Götz* (2002) are still in discussion. Hence, the objective of the present study was the comparison of these methods with a full multitrait animal model. This was done in a stochastic simulation study mimicking a simplified breeding scheme of Austrian Brown Swiss cattle. Special attention was also put on assuming different residual covariances.

MATERIAL AND METHODS

A population structure roughly reflecting the Austrian Brown Swiss cattle population was simulated with the stochastic simulation program ADAM (*Pedersen et al.,* 2009). Approximately 51,300 cows distributed on 1,710 herds were simulated. Five traits following Gaussian distribution were chosen to represent the blocks dairy (fat (FY) and protein (PY) yield), beef (net daily gain (NDG)) and fitness (somatic cell count (SCC) and non-return rate (NRR) of cows). Further requirements were a wide range of heritabilities and genetic correlations as well as economic importance. Four traits FY, PY, SCC and NRR were measured on all female animals, NDG was observed on approximately 60% of all male animals. Each trait was measured on every animal in all herds, no repeated records were assumed. The assumed heritabilities and genetic correlations for the five traits are shown in *Table 1.* Around 25% of young bulls and 75% of proven bulls were used for matings in the selection scheme. Breeding values and phenotypes for the five traits were simulated for base population animals. Afterwards animals were selected on a TMI based on multivariately estimated breeding values (EBV) over 30 years. Relative economic weights for FY, PY, NDG, SCC and NRR were adopted from the values used in routine genetic evaluation, which are 5.4, 53.6, 4.3, 19.7 and 17% respectively (*Fuerst et al.,* 2013). Three different scenarios with respect to the covariances of the residual effects were simulated. In scenarios 0, 1 and 2 residual correlations were varied from zero, to half and equal to the genetic correlations, respectively. The variation of the residual covariances was specifically evaluated to appraise the impact of ignoring residual covariances. In total, ten replicates were conducted for each scenario.

Table 1

Heritabilities (on the diagonal) and true genetic correlations (above diagonal)

Trait	FY	PY	NDG	SCC	NRR
FY	0.40	0.85	0.10	0.25	-0.20
PY		0.39	0.10	0.25	-0.20
NDG			0.27	0.00	0.00
SCC				0.12	-0.10
NRR					0.02

Method A was a full multitrait animal model based on raw data using the true genetic and phenotypic parameters. The model included a fixed herd-year-effect, a random genetic and a random residual effect. Subsequently the TMI was calculated as:

$$\mathbf{TMI_A = EBV_{FY}\,\omega_{FY} + EBV_{PY}\,\omega_{PY} + EBV_{NDG}\,\omega_{NDG} + EBV_{SCC}\,\omega_{SCC} + EBV_{NRR}\,\omega_{NRR}} \quad (1)$$

where **EBV** refers to the certain traits; **ω** denotes the relative economic weights which are 5.4% for FY, 53.6% for PY, 4.3% for NDG, 19.7% for SCC and 17% for NRR, respectively. Method A was considered to be the reference method. For methods B, C and D, EBVs were estimated in univariate animal models including the same effects described above. In order to obtain the TMI of method B, which is the currently used method (proposed by *Miesenberger,* 1997), C (proposed by *Dempfle; Götz*, 2002) and D (proposed by *Reinhardt*; *Götz*, 2002) equation (1) was applied and covariances between the EBVs (σ_{ij}) of the different methods (indicated by sub-indices B, C, D) were calculated as:

$$\mathbf{\sigma_{ijB} = r_{gij} r^2_i r^2_j \sigma_{ai} \sigma_{aj}} \quad (2)$$

$$\mathbf{\sigma_{ijC} = r_{pij} r_i r_j \sigma_{ai} \sigma_{aj}} \quad (3)$$

$$\mathbf{\sigma_{ijD} = r_{gij} r_i r_j \sigma_{ai} \sigma_{aj}} \quad (4)$$

where $\mathbf{r_{gij}}$ is the genetic correlation between traits i and j; $\mathbf{r^2_{i,j}}$ are the reliabilities of EBVs of traits i and j;$\mathbf{\sigma_{ai,j}}$ are the additive genetic standard deviations of traits i and j; $\mathbf{r_{i,j}}$ are the accuracies of EBVs of trait i and j and $\mathbf{r_{pij}}$ is the phenotypic correlation between traits i and j.
This means that only method C accounts for residual correlations.

Estimated breeding values were calculated using the program package MiX99 (*Lidauer et al.,* 2013). For all methods, genetic parameters were not re-estimated. The true (simulated) simulated parameters were used. All EBVs were standardised to 12 points per additive genetic standard deviation. The base was set to 100 for the years 18 to 22.

RESULTS AND DISCUSSION

Across all year groups Spearman rank correlations between the true and the estimated breeding values were about 0.86 for scenario 0 and about 0.83 for scenario 2. For scenario 1, which is not shown in *Table 2*, the correlation across all year groups is above 0.86.

As in scenario 2 genetic and phenotypic correlations are identical, the results for methods C and D are the same. Rank correlations between true and estimated TMIs across year groups are moderate, because of relatively low reliabilities in the simulated population (approximately 41% of the simulated animals have a r^2 below 60%). Rank correlations within year groups are rather similar, but slightly lower for method B in scenarios 1 and 2. Rank correlations of scenario 2 are in general slightly lower than the correlations of scenarios 0 and 1. Furthermore rank correlations between the full multivariate method (A) and all other methods, including all scenarios were calculated. Rank correlations are in the range of 0.93 to 0.99 within year groups. Across all animals rank correlations are between 0.98 and 0.99.

Table 2

Rank correlations between the true TMI within year groups for different methods for scenarios 0 and 2

Scenario	Years	A	B	C	D
0	All	0.8704	0.8612	0.8606	0.8620
	11–15	0.6399	0.6149	0.6054	0.6122
	16–20	0.6516	0.6283	0.6244	0.6258
	21–25	0.6262	0.5923	0.5953	0.5980
	26–30	0.6657	0.6343	0.6406	0.6404
Scenario	**Years**	**A**	**B**	**C**	**D**
2	All	0.8490	0.8346	0.8476	0.8476
	11–15	0.6274	0.5948	0.6233	0.6233
	16–20	0.6237	0.5890	0.6196	0.6196
	21–25	0.5790	0.5288	0.5745	0.5745
	26–30	0.6190	0.5785	0.6151	0.6151

In this study biases are products of subtracting the true TMI from the estimated TMI. This was done for all animals and scenarios. *Table 3* shows the bias of scenarios 0 and 2. Results for scenario 1 are between scenario 0 and 2.

Table 3

Bias of different TMI methods from the true TMI within year groups for scenarios 0 and 2

Scenario	Years	A	B	C	D
0	All	-0.1	0.1	0.1	0.2
	11-15	-0.4	-1.5	-1.2	0.4
	16-20	-0.1	-0.4	-0.4	0.1
	21-25	0.1	0.7	0.6	0.1
	26-30	0.1	1.5	1.2	0.1
2	All	-0.1	-0.6	-0.1	-0.1
	11-15	-0.3	-3.0	-0.7	-0.7
	16-20	-0.1	-0.8	-0.2	-0.2
	21-25	0.0	0.8	0.2	0.2
	26-30	-0.1	0.7	0.2	0.2

Results of scenario 0, where no residual covariances were assumed, show very good results particularly for methods A and D. Methods B and C seem to underestimate the animals in the first years in both scenarios. One possibility can be an incomplete pedigree and the use of phantom parents groups (*Fuerst et al.,* 2014). However, method B leads to an overestimated genetic trend. This trend is more pronounced when residual covariances are assumed. This overestimation is even stronger in the best 10% animals in TMI per year. *Table 4* shows the bias (EBV-TBV) of the TMIs of the top 10% animals within year groups.

Figure 1 shows the bias for the top animals in scenario 1, which is expressed as a downwards bias in the first years and an upwards bias in the last years.

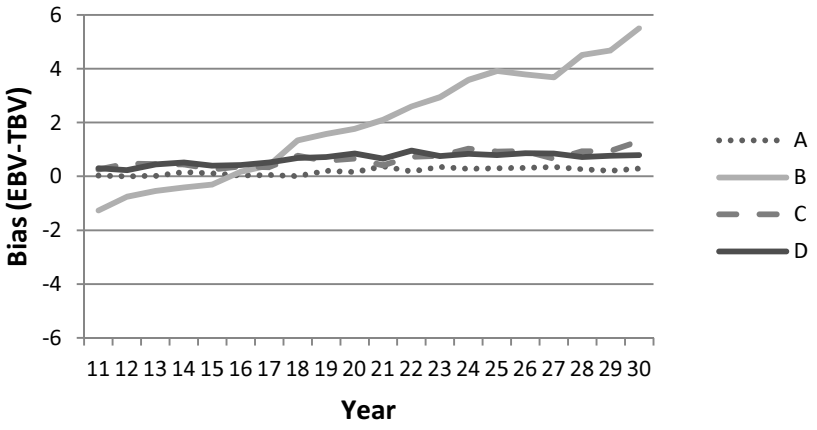
Table 4

Bias of different TMI methods from the true TMI for the top 10% within year groups for scenarios 0 and 2

Scenario	Years	A	B	C	D
0	All	0.2	1.5	1.7	1.0
	11-15	-0.2	-0.6	0.3	1.0
	16-20	0.1	0.4	1.0	0.9
	21-25	0.4	2.2	2.3	0.9
	26-30	0.5	3.8	3.1	1.0
2	All	0.2	2.3	0.6	0.6
	11-15	-0.2	-0.4	-0.2	-0.2
	16-20	0.2	1.7	0.5	0.5
	21-25	0.4	3.7	1.0	1.0
	26-30	0.4	4.1	1.0	1.0

Figure 1

Time trend of bias (EBV-TBV) of different methods for the top 10% animals within years for scenario 1



CONCLUSIONS

Results show that all methods based on selection index theory are quite similar. The analysed methods show good results when residual covariances are zero. However, in real data residual covariances can have an important impact. It is well known that omitting residual covariances when the same animals are recorded in the same environment is not valid. The currently used method B shows good results for high

reliabilities but leads to inflated deviations mainly in case of low reliabilities. This results in a bias particularly for the top animals and can therefore be relevant in terms of selection accuracy. For the joint genetic evaluation of Austria and Germany, it is intended to replace the current method of TMI calculation by a multitrait approach. If this is not working, an adapted method of including residual covariances between traits is needed.

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Optimizing alternative schemes of community-based breeding programs for two Ethiopian goat breeds

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ABSTRACT

One tier community-based breeding schemes with four different scenarios for Western Lowland and Abergelle goat of Ethiopia were simulated using ZPLAN computer program to compare the genetic gain of the breeding objectives traits across the alternatives. The scenarios were different in terms of numbers of traits in selection criteria while keeping the breeding goals the same throughout the alternatives. Simulation study showed that there was little difference in annual genetic gain for individual breeding objectives traits and annual monitoring gain (aggregate gain) across the different alternatives. The range of 0.8702–0.8724 kg, 0.0001–0.0006 and 0.0184–0.0195% of six months weight, number of kids born and proportion of weaned kids were predicted for Western Lowland goat respectively. For Abergelle goat the range of 0.36–0.3675 kg of six months weight, 0.0066–0.0114 kg of daily milk yield and 0.0068–0.0085% proportion of weaned kids per does per year were simulated. The lowest aggregate genetic gains of 25.94 and 16.42 were obtained for Western Lowland and Abergelle goat respectively from the alternative where only one trait (growth) included in the selection criteria (index or record).
(Keywords: Breeding program, Ethiopia, Genetic gain, Goat, selection criteria)

INTRODUCTION

Almost all goats in Ethiopia are managed by resource poor smallholder farmers and pastoralists under traditional and extensive production systems. They provide multiple roles for their owners such as source of income, food (meat and milk) and manure (*Legesse et al*., 2008; *Abegaz et al*., 2013). They also serve as a means of risk mitigation during crop failures, property security, monetary saving and investment in addition to many other socioeconomic and cultural functions (*Negassa and Jabbar,* 2008). The growing demand of meat at the domestic as well as at the international markets also increases the importance of goat in the national economy of the country. However, goat production in Ethiopia is constrained by many biological, environmental and socio-economical factors. Among them, lack of systematic breeding programs is an important constraint.

There is no systematic goat breeding program in place and goat is the most neglect livestock species in research and development endeavors (*Tsegahun et al*., 2000). There have been a few attempts of genetic improvement program of goats through upgrading the exotic genetic blood levels. However it was reported that crossbred goats did not perform better than indigenous goats if both groups were kept atsimilar management levels (*Ayalew et al*., 2003). In general, many small ruminants cross breeding programs in tropical country were not successful because of the incompatibility of the genotype with the farmers breeding objectives, management methods and the prevailing environment of the tropical low input production systems (*Ayalew et al*., 2003; *Kosgey et al*., 2006). Pure breeding applying community based breeding program is believed to be a more appropriate breeding program for such type of production systems which are characterized as low-input system with poorly developed infrastructures (*Sölkner et al*., 1998; *Gizaw et al*., 2009). Optimization of the community based breeding programs by looking at different alternative schemes to predict the genetic gain and the economic return is very helpful during implementation. It gives the chance to adjust the technical, infrastructural and socio economic issues ahead of the implementation. The objective of this study is to evaluate different alternative schemes of community-based breeding programs for Abergelle and Western Lowland goat breeds of Ethiopia.

MATERIAL AND METHODS

Study areas

The study was conducted in two districts (Metema and Abergelle) of the Amhara Regional State of Ethiopia. Metema is located at about 860 km North West of the capital Addis Ababa. The district has an altitude of 550 to 1608 m and the latitude of 12°40' N to 13°14' N. It has uni-modal type of rainfall receiving the annual average of 850 to 1100 mm, which occurs from June to September. The production system is characterized as mixed crop-livestock system with crop dominance. Western Lowland (Gumuz) breed is the dominant goat breed of the area. Abergelle is located at about 780 km from Addis Ababa in the Northern part of the country. The area is characterized as dry/sub-moist highland agro-ecological zone. It has the altitude of 1150 to 2500 m with the latitude of 12°18'N to 13°06'N. The rain fall pattern of the area is very erratic and uneven. The area receives the mean annual rainfall ranges from 250 to 750 mm. The main rain season of the district is July to September. The production system of Abergelle district is mixed crop-livestock system with high priority of Abergelle goat production.

Breeding objectives and selection criteria

As the breeding program would be implemented at community level, for each breed, only three traits with high preference by farmers and easy to measure were considered (*Abegaz et al*., 2013; *Abegaz,* 2014). The breeding objectives identified for Abergelle goat owners were: Body size, milk yield and mothering ability (kids survival), while the breeding objectives for Western Lowland goat owners were body size, twinning rate and mothering ability (kids survival). Two selection indexes, one for each breed were constructed. Index 1, to reflect the breeding objective of Abergelle goat breeders, included six months weight (for body size), daily milk yield (for milk yield) and proportion of kids weaned (for kid survival). Index 2 to reflect the breeding objective of Western Lowland goat six months weight, number of kids born per does per year and proportion of kids weaned per does per year.

Economic values

The economic weight and variance components of the traits are given in *Table 1*. The relative economic weight is derived based on the farmers' preference through participatory methods (*Abegaz*, 2014). The phenotypic standard deviations of the traits were estimated from the result of morphological characterization studies of the breeds (*Abegaz et al*., 2013). The genetic standard deviations of the traits were also estimated by multiplying the phenotypic standard deviation to the heritability of a trait.

Table 1

Economic weight and variance component of the selection criteria (traits)

Breeding objective traits	Selection criteria	Unit	REW	σ_a	σ_p
Abergelle					
Body size	Six month weight	kg	54%	1.45	2.74
Milk yield	Milk yield/ day	kg	30 %	0.13	0.23
Kid survival (mothering ability)	Proportion of kids weaned/does/year	%	16%	0.089	0.40
Western Lowland					
Body size	Six months weight	kg	55%	1.99	3.76
Twinning	Number of kid born /doe/year		31%	0.14	0.45
Kid survival (mothering ability)	Proportion of kids weaned /does/year	%	14%	0.13	0.60

REW: relative economic weight; σ_a: Additive genetic standard deviation; σ_p: phenotypic standard deviation

Population structure

The community based one tier selection scheme was considered for both breeds as the optimal breeding program for both of the study areas. The flocks from 30 households with the average of 26 breeding does per household were considered as one breeding unit for Abergelle goat, while the flocks from 60 households with the average of 5 breeding does per household was considered as one breeding unit for Western Lowland goats. The important input parameters of the two breeds for modeling (running ZPLAN) are shown in *Table 2*. The information for the input parameters were taken from the previous studies (*Derbie*, 2008; *Abegaz et al*., 2013, *Derbie and Taye*, 2013; *Abegaz*, 2014). The number of proven (candidate) animals in each time unit (year) were projected using the reproductive parameters and survival rate of the breeds. In this study, only the costs of additional activities to the normal management practices were considered as the cost parameters.

Alternatives breeding programs

Four different alternatives for each breed were proposed for evaluating optimal breeding program (*Table 3*). The alternatives were based on the variation of the number of the traits in the selection index (recording) while keeping all traits in aggregate breeding goal. The important considerations of the alternatives were to see the effect of the variation of the number of traits in the recording scheme (selection criteria) on the genetic gains of the individual traits as well as the aggregate response.

Table 2

Input parameters for modeling alternative breeding programs

Parameters	Abergelle	Western Lowland
Population parameters		
Population size (Does)	780	300
Number of proven males/years	300	255
Proportion of bucks selected	10%	10%
Biological parameters		
Breeding does in use (year)	5	5
Breeding bucks in use (year)	2	2
Mean age of bucks at birth of first offspring (years)	1.5	1.2
Mean age of does at birth of first offspring (years)	1.3	1.1
Kidding rate	0.85	0.85
Mean time period between subsequent kidding (years)	1	0.6
Mean number of kids per litter (litter size)	1.13	1.5
Number of kidding/doe/year	1	1.67
Kid survival to six months (%)	80%	80 %
Cost parameters		
Animal identification doe/year(€)	0.86	1.36
drug /doe/year(€)	0.86	1.36
Enumerator salary(€)	0.98	0.98
Stationary materials for recording(€)	0.20	0.20
Interest rate return (%)	0.05	0.05
Interest rate cost (%)	0.08	0.08
Investment period /year	15	15

Table 3

Alternative breeding schemes for Abergelle and Western Lowland goats

Alternatives	Breed	
	Abergelle	Western Lowland
1	All traits in the selection index (SMW+DMY+PKW)	All traits in the selection index (SMW+NKB+PKW)
2	SMW+DMY in the selection index	SMW+NKB in the selection index
3	SMW+PKW in the selection index	SMW+PKW in the selection index
4	Only SMW in the selection index	Only SMW in the selection index

Note: SMW=Six months weight, DMY=Daily milk yield, PKW=Proportion of kid weaned, NKB=Number of kids born

Genetic and phenotypic parameters

The genetic and phenotypic parameters are presented in *Table 4*. Due to the population parameters of the study breeds lacking, the weighted heritability estimates of the traits from published reports of other local and exotic goats were used. The genetic and phenotypic correlations of the traits were obtained from published reports on sheep.

Table 4

Phenotypic correlation (above the diagonal), genotypic correlation (below the diagonal) and heritability of the traits (along diagonal)

Traits	Abergelle			Western Lowland		
	SMW	DMY	PKW	SMW	NKB	PKW
SMW	0.28	0.1	0.1	0.28	0	0.1
DMY/NKB	0.2	0.32	0.14	0	0.10	0.15
PKW	0.3	0.53	.05	0.3	-0.20	0.05

Note: SMW=Six months weight, DMY=Daily milk yield, PKW=Proportion of Kids weaned, NKB=Number of kids born

Evaluation of alternative breeding programs

Alternative breeding schemes were evaluated using the computer program ZPLAN (*Willam et al*., 2008). Using the gene flow method and selection index procedures, the program enables to simulate different breeding plans by deterministic approach. The program calculates genetic gain for the aggregate breeding value, the annual response for each trait and discounted return and discounted profit for a given investment periods. Rate of inbreeding per generation (ΔF) were calculated using a formula relating effective population size to use number of male (N_m) and number of female (N_f) breeding animals (*Falconer and Mackay*, 1996); $\Delta F = (1/8\ N_m) + (1/8\ N_f)$

RESULTS AND DISCUSSION

Annual genetic gain in individual traits

The predicted annual genetic gains (ΔG) of individual breeding objectives traits from different alternative schemes of the two breeds are presented in *Table 5*. Those parameters were different among the different alternatives and breeds. For all traits considered, higher genetic gains were predicted for Western Lowland goats than the Abergelle goats. These variations were due to higher phenotypic variation of the traits, lower generation interval and better performance (such as high twinning rate) of Western Lowland goats. The highest genetic gain of 0.3676 kg per year for six month's weight was predicted for Abergelle goats in growth only scheme (alternative 4) while the lowest 0.3599 was obtained in the alternative 2. As expected the highest gain was simulated for six month weight from growth only alternative where only the information of growth was included in the selection index. The highest value 0.8724 kg annual genetic gain of the six months weight was simulated for Western Lowland goats from alternative 3 (growth and survival information in the selection index) whereas the lowest value of 0.8702 kg was simulated from alternative 2 (growth and twinning information in the selection index). The highest gain of six month weight from alternative 3 was due to relatively higher positive genetic and phenotypic correlation between the two traits. The lowest genetic gain of six months weight from growth and twinning alternative was associated with the lower phenotypic and the negative genetic correlation of the two traits attached in the model. The genetic gain of six months weight predicted in this study is in the range of the predicted annual genetic gain of six months weight in similar study of Kenyan cross breed goats (*Bett et al*., 2012).

Table 5

Genetic gain per year for the breeding objective traits in different alternatives

Breed	Alternativies	Traits			
		SMW(kg)	DMY(kg)	PKW (%)	NKB
Abergelle	1 SMW+DMY+PKW	0.3600	0.0114	0.0085	_
	2 SMW+DMY	0.3599	0.0110	0.0083	_
	3 SMW+PKW	0.3669	0.0069	0.0072	_
	4 SMW	0.3675	0.0066	0.0068	_
Western Lowland	1 SMW+NKB+PKW	0.8710	-	0.0192	0.0006
	2 SMW+NKB	0.8702	-	0.0184	0.0006
	3 SMW+PKW	0.8724	-	0.0195	-0.0001
	4 SMW	0.8718	-	0.0186	0

SMW: Six months weight, DMY: Daily milk yield, NKB: Number of kids born, PKW: Proportion of kids weaned

Relatively lower genetic gains of 6.60 g and 6.97 g milk yield were predicted from alternatives 4 and 3 for Abergelle goats, respectively. Higher values of 11.43 and 11.37 g of milk yield were predicted from alternatives 1 and 2, respectively. In these alternatives the information of milk yield was included in the selection index. Differently from this result higher genetic gain 0.261–0.809 kg milk yield were predicted in different alternatives of Kenyan dairy goat (*Bett et al*., 2012). However, a very close result with the range of 0.018–0.020 kg of genetic gain of milk yield was predicted for different alternatives in a study on Ethiopian Afar sheep (*Mirkena et al*., 2012). There was a difference of 4.77 g in genetic gain of milk yield between the alternative with highest gain and the alternative with the lowest gain in the present study. This result indicates that including milk record in the selection index would result the positive genetic gain but the profit will be minimal. Milk recording at village level is operationally difficult and routine milk recording even at monthly intervals is costly. It may be more appropriate to rely on indirect selection of milk yield through associated traits in this situation.

The genetic gains of kid survival at different scenarios ranged between 0.006764% to 0.008517% for Abergelle goat, while it ranged from 0.018389% to 0.019227% for Western Lowland goats. In both breeds, the differences of annual genetic gain of kid survival between different alternatives were very small. This is because of the low heritability of the trait and low correlation with other traits. Comparable results with the range of 0.00–0.007% were predicted from different alternatives for Kenyan dairy goat breeds (*Bett et al*., 2012) and the range of 0.009–0.01% for Ethiopian Afar sheep breed (*Mirkena et al*., 2012).

Very low genetic gains of twining rate were predicted from all alternatives for Western Lowland goats. Even negative gain was predicted from the alternative 3 and 4 where the twinning information was not included in the recording scheme. This is due to the low heritability of the trait and low phenotypic and genetic correlation with other traits. In addition to this, selection intensity was mostly derived from the male path of selection thus the twinning rate performance information was obtained only from the dams of young bucks. Since recording of the twinning rate is very simple, it would be worthwhile to include the information of twinning rate in the recording and give more

weight in breeding goal to avoid the loss of genetic gain of twinning rates which was reported as the most preferred traits in Western Lowland goat keepers.

Evaluation criteria

Table 6 depicts the important evaluation criteria simulated by ZPLAN program. The selection accuracies of obtained from different alternatives for both breeds were in the acceptable range 0.481 to 0.512. Relatively higher accuracy of selection 0.504 and 0.512 were obtained from Alternative 1 (all traits in selection index) for Abergelle goats and Western Lowland goats, respectively. This reflects as the information source increased in the selection criteria the accuracy also increased. The annual monetary genetic gains ranged between 16.42 to 17.57 Euro/doe for Abergelle goats from the different alternatives whereas 25.96 to 26.06 Euro were predicted for western Lowland goats. As the difference between the schemes was only by varying the information source in the selection index, there was no difference between the different alternatives in selection intensity and generation interval within the same breed. The differences of those parameters between the two breeds were connected with the difference of population size of the breeding does and the difference in reproductive performance of the breeds in input parameters. A selection intensity of 1.99 and a generation interval of 2.88 years were predicted for Abergelle goats while the corresponding values for Western Lowland goats were 2.25 and 2.14 years. The discounted profit found in all alternatives and in both breeds was very high. It might not be appropriate to compare the alternatives in this study based on the discounted profit because the economic value attached to each trait is not in the real monitoring term and only additional cost to the normal practice were considered as the cost. The relative economic weights based on farmers' preference were assigned as the economic weight. The rate of inbreeding per generation 0.4% and 1.3% were calculated for Abergelle and Western Lowland goats respectively. The higher inbreeding rate for Western Lowland goats could be explained by the small flock size per household. During the implementation period, increasing the participant farmers within the village or implementing across village selection for Western Lowland goat breeds would be advisable to avoid the problem of inbreeding.

Table 6

Important evaluation criteria simulated from different alternative in Abergelle and Western Lowland goats

Alternative	Criteria	Abergelle	Western Lowland
1	Accuracy of selection	0.503	0.512
	AMGG	17.57	26.06
	Discounted profit/doe	138.85	213.29
2	Accuracy of selection	0.504	0.511
	AMGG	17.51	26.05
	Discounted profit/doe	138.48	212.83
3	Accuracy of selection	0.484	0.511
	AMGG	16.58	26.01
	Discounted profit/doe	133.24	212.99
4	Accuracy of selection	0.481	0.510
	AMGG	16.42	25.93
	Discounted profit/doe	132.32	212.41

AMGG: Annual Monitoring Genetic Gain

CONCLUSION

The community level alternative schemes were designed and predicted for smallholder goat farmer conditions. Community based breeding program is the breeding program implemented at the smallholder levels where the infrastructure is poor and low input production system prevails. Therefore, the organizational structure should be simple and the traits in the recording should also be small in number to avoid complexity during implementation (*Sölkner et al*., 1998; *Wurzinger et al*., 2008; *Gizaw et al*., 2009). This study was aimed to see how much genetic gain and economic return in aggregate breeding goals (breeding objectives traits) can change by varying the number of traits at selection criteria. Even though, relatively higher gain from the alternatives with more traits in the selection criteria, the magnitude of the loss in genetic gains and economic returns from the alternatives with single versus more traits in the selection index were very small. For instance, the difference in annual monitoring genetic gain between all traits and one trait alternative for Abergelle goats were 1.154%. This indicates that it is possible to start a feasible community based breeding with growth only or very few traits in selection criteria with little loss of genetic gain in breeding goal traits.

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Sustainability of intensive beef production system in North-East Italy: relationships between phosphorus supply and productive performance

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ABSTRACT

The beef sector of the Veneto Region is based on young bulls imported mainly from France and reared intensively using total mixed rations based on maize silage and concentrates. While nitrogen excretion of the sector is regulated by Nitrate Directive, the excretion of phosphorus (P) is less studied, despite of its potentially great impact on environment. This study aims at analysing the relationships between productive and economic performances and P content of the diet in 14 farms of the region. For a whole productive year feed consumption, ingredients and chemical composition of diets were monthly collected. Average Daily Gain (ADG), Feed conversion ratio (FCR), daily gross profit (DGP), and P balance were calculated. ADG, FCR, and DGP were analysed with a mixed model using arrival season, arrival weight, class of dietary content of P, protein and starch as fixed effects and farm as random effect. Average daily gain was 1.39±0.08 kg/d, FCR was 0.14±0.01 kg/kg, and DGP 2.5±0.40 €/d. The P dietary content was on average high (0.38±0.04, % DM), which resulted in P intakes and excretions of 13.49±1.94 and 9.85±1.92 kg/head/place, respectively. None of the productive and economic traits was affected by phosphorus content of the diet. As a consequence, the phosphorus supplementation can be reduced without the risk of weakening productive and economic performances.
(Keywords: Beef cattle, intensive farms, environment, Charolais breed, P excretion)

INTRODUCTION

Livestock production has complex interactions with the natural environment, especially for nitrogen (N) and phosphorus (P) excreted by animals (*Gerber et al.,* 2013). In the recent years, the research has mainly focused on human-related nitrogen impacts on environment, whereas phosphorus impacts have been less studied (*Schipanski and Bennet,* 2012).

The beef sector of the Veneto Region (North-East Italy) represents an important contributor to the national beef production. It is based on young bulls imported mainly from France (especially Charolais breed), and intensively reared for 7-8 months using total mixed rations (TMR). The most important feeds used are maize silage and concentrates (*Xiccato et al.,* 2005). In the last years, the sector has met the European Union's thresholds about nitrogen application on agricultural fields imposed by the Nitrate Directive (n.676/92). However, although manure application is carried out to meet crops N requirements, and also to respect the EU nitrogen thresholds, P surpluses

in soils are observed, since the N/P ratio required by plants is higher than that in manure (*Kissinger et al.,* 2005).

Due to the affinity of phosphorus compounds with soil's elements (*James et al.,* 1996), and the practice to concentrate the application of manure nearby their production sites in intensive farming systems (*Defra,* 2004), these surpluses have led to soil P accumulation in various areas of Europe (*Hooda et al.,* 2001*; Ott and Rechberger,* 2012). The resulting reduction of soil capacity to adsorb phosphorus could cause an increase of leaching rates to groundwater bodies (*Pautler and Sims,* 2000), and also of phosphorus loss with runoff events, carrying to a greater eutrophication risk of surface water resources (*James et al.,* 1996).

Overfeeding of beef cattle with P is common in the practice, partly because is frequent the inclusion in the diet of feeds naturally high in P, partly because additional P supplementation may occur irrespective of the actual P content of the diets (*Vasconcelos et al.,* 2007). This study aimed to analyse the effects of phosphorus supply on animal productive performances in the North-East Italy intensive beef sector, in order to evaluate whether P excretion could be reduced without consequences on productive and economic performances.

MATERIAL AND METHODS

Data for this study originated from 14 specialized fattening herds located in the Veneto region and associated to AZoVe (Associazione Zootecnica Veneta, Ospedaletto Euganeo, Italy), a large cooperative of beef producers. The reference unit for data collection was the batch, defined as a group of stock calves homogeneous for genetic type, origin, finishing herd and fattening period. For each batch the following data were acquired: average BW at arrival and at sale (kg); fattening length (d); purchase and sell price per head (€/head). These data were used to compute the following traits: average daily gain (ADG, kg/d), calculated as (live weight at sale – live weight at arrival)/fattening length; feed conversion ratio (FRC, kg/kg), calculated as (live weight at sale – live weight at arrival)/total feed DM intake in fattening period; daily gross profit (DGP), calculated as value at sale - value at purchase, and expressed per day of fattening (€/d). Herds were visited monthly during the whole year, diet formulations and a sample for TMR were collected for each batch, and the weight of total mixed ration (TMR) uploaded into the manger for each batch was recorderd. Two subsequent intake observations were averaged to obtain the mean daily dry matter intake (DMI). Diets were chemically analysed for determination of dry matter (*AOAC method 934.01*, 2003), crude protein (*Kjeldahl, AOAC method 976.05*, 2003), ash (*AOAC method 942.05*, 2003), Neutral Detergent Fiber according to *Van Soest* (1991), starch (HPLC method; *Bouchard et al.*, 1988) and phosphorus content (*AOAC 999.10*, 2000 and ICP-OES).
Only batches with Charolais breed and more than four month samples were considered in the study. The final data set included 126 batches, 8545 animals and 105 diets.

Phosphorus balance
Phosphorus balance was calculated following the ERM method (2001). The model estimates P excreted as P intake – P retention. Each element refers to 1 head/batch/year. The single elements are obtained as follows:

P intake = Intake* (P diet/100) (kg), where Intake is the total feed intake for head/batch/year

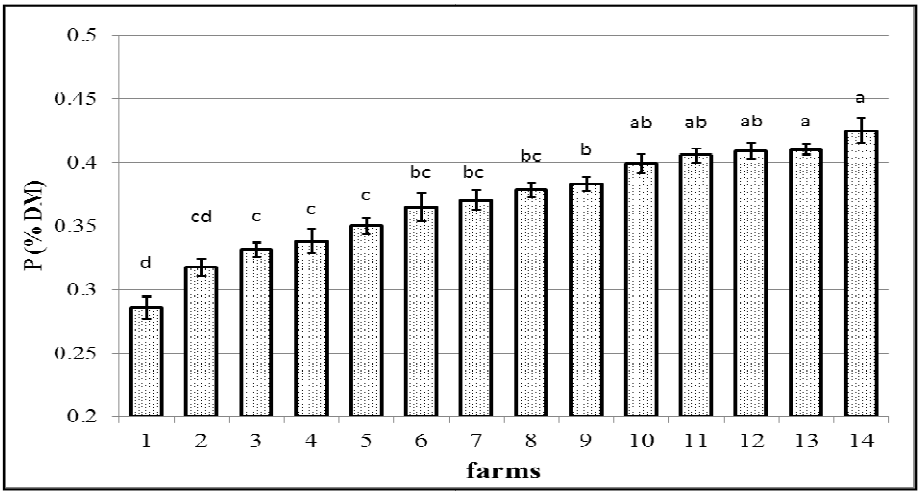
P retention = (LWf – LWi) * K_P (kg), where LWf and LWi are final and initial live weight respectively, and K_P is phosphorus retention per life weight unit coefficient, corresponding to 0.0075 kg/kg (*Whiters et al.,* 2001).

Statistical analysis

For statistical analysis, the database was edited as follows: the P content (% DM) of the diet was grouped in three classes (ClP) on the basis of 25th and 75th quartile; the same procedure was used for protein (ClPr) and starch (ClS) content. Season of arrival was classified as winter, spring, summer, autumn on the basis of arrival month of the batch; arrival weight was divided into three classes based on the mean±1SD. A preliminary analysis (GLM) showed a large variability among farms in P dietary content (Figure 1). The P content was correlated (r=0.41, P<0.001) with the proportion of feeds used to increase N dietary content (mix of oilseed by-products, corn distiller and maize gluten feed, and a commercial mineral-protein supplement), and the variability among farms can be explained with different feeding strategies and management practices. For this reason, we decided to use the farm as random effect in the final statistical model.

Figure 1

LSmeans for P content of diet (% DM) for farms



Average daily gain (ADG), feed conversion ratio (FCR), and daily gross profit (DGP) were analysed with mixed linear models (SAS, 1991), with arrival season, arrival weight class, protein class (ClPr), starch class (ClS), and P class (ClP) as fixed effect and farm as random effect.

RESULTS AND DISCUSSION

Mean initial and final body weight were 390 and 714 kg, respectively (*Table 1*), and the fattening period averaged 233 d. In this period, the mean daily DMI resulted of 10.2 kg/d, ADG was 1.39 kg/d, and FCR was 0.14 kg/kg, with a range of variation among batches wider for ADG than for FCR.

Table 1

Descriptive statistics for productive performances

Item	Unit	Mean	SD	Min	Max
Initial live weight	Kg	390.4	28.44	322.0	458.0
Final live weight	Kg	714.2	20.34	670.0	772.0
Duration	D	233	18	190	324
DMI	kg/head/d	10.22	0.79	8.27	11.73
ADG	kg/d	1.39	0.08	1.19	1.60
FCR	kg/kg	0.14	0.01	0.11	0.17
Daily gross profit	€/d	2.50	0.40	1.66	3.39

DMI: dry matter intake; ADG: average daily gain; FCR: feed conversion ratio

The ADG found was similar to those obtained for Charolais breed reared in Veneto Region (*Sturaro et al*., 2005). Moreover, ADG and FCR mean values were similar to those obtained in performance experiments using maize-based diets (*Mandell et al.*, 1997; *Arthur et al.*, 2001). About economic result, DGP was 2.50 €/d on average. A relevant variation among batches was recorded, with the maximum value being almost double than the minimum. A positive correlation existed between ADG and DGP ($n=123$, $r=0.56$, $P<0.001$).

The TMRs of all the batches contained maize silage and soybean meal, and almost all contained also maize flour (89% of TMRs) and sugarbeet pulp (83% of TMRs); corn distiller, maize gluten feed, alfalfa hay, wheat straw, hydrogenated fat and mineral-protein supplement completed the mean diet; other ingredients were less important (data not shown).

The mean chemical composition of diets is shown in *Table 2*. Mean phosphorus level resulted 0.38% DM, with a relevant variability since the highest TMR content was 1.7 times the lowest one.

Table 2

Descriptive statistics for chemical composition of diet (% DM)

Item	Mean	SD	Min	Max
P	0.38	0.04	0.27	0.45
CP	13.86	0.74	11.48	15.55
Ash	5.93	0.37	5.06	6.62
Starch	33.89	3.90	27.06	42.74
NDF	32.03	2.98	24.31	38.23
NSC	44.75	3.20	38.54	53.05

CP: crude protein; NDF: neutral detergent fiber; NSC: not structural carbohydrates

The range of P dietary contents observed is higher, even in the lowest values, than the reference P requirements for beef NRC (2000), 27.6–52.7 g P/d observed vs 21–22 g P/d recommended, probably because of the practice of including P supplementation in the protein supplement without accounting for the basal diet content. Protein levels were on

average 1.42 kg/d, higher than the NRC (2000) recommendations of 1.07 kg/d CP, although also in this case there was a remarkable variability (range: 11.5−15.5% DM). Finally, contents of starch, which is an important source of energy for fattening young bulls varied from 27 to 43%.

The results of P balance are given in *Table 3*. The intake, depending on the combination of varying DM intakes and P (%DM) contents, varied more than the retention, which depended on a moderately variable growth rate. The resulting P excretion was close to 10 kg/head/d, with a wide variability; the same value, expressed as kg/day/1000 heads, was higher than that found for US intensive beef production (28.1 vs 23.1 kg) (*Cole and Todd*, 2009). Phosphorus efficiency was in the lowest values of the ranges reported in literature, and highy variable.

Table 3

Descriptive statistics for phosphorus balance

Variables	Unit	Mean	SD	Min	Max
P intake	kg/head/y	13.49	1.94	9.65	18.45
P retention	kg/head/y	3.64	0.21	3.12	4.20
P excretion	kg/head/y	9.85	1.92	6.05	15.11
P efficiency	%	27.55	4.20	18.09	37.31

The results of statistical analysis of ADG, FCR, and DGP are given in *Table 4*. The effect of arrival season was statistically significant for all variables, as expected from what usually observed in the practice of this fattening system: ADG, FCR and DGP were higher for batches arrived in summer than in winter (ADG: 1.41 and 1.36 kg/d; FCR: 0.14 and 0.13 kg/kg; DGP: 2.72 *vs* 2.2−2.1 €/d).

Table 4

Mixed model analysis for productive performances

Effect	ADG (kg/d)		FCR (kg/kg)		DGP (€/d)	
	F	P-value	F	P-value	F	P-value
Arrival Season	4.51	0.01	12.76	< 0.01	34.84	< 0.01
Arrival weight	3.41	0.04	4.23	0.02	1.75	0.18
ClP	0.43	0.65	1.20	0.31	0.90	0.41
ClS	3.18	0.05	2.26	0.11	3.90	0.02
ClPr	0.43	0.65	2.84	0.06	2.73	0.07
RMSE	0.05		0.01		0.25	

ClP: classes of P (%DM), ClS: classes of starch(%DM), ClPr: classes of protein (%DM)

Effects of arrival weight were less marked, and significant only for ADG, which decreased with increasing weight class (1.42 to 1.36kg/d, respectively for the light and heavy classes) and FCR, which observed the same trend with increasing weight class (0.14 to 0.13 kg/kg, respectively for the light and heavy classes). This was also expected since young bulls lower at arrival tend to grow faster (*Chambaz et al*., 2001). The levels

of P had no effect on ADG, FCR, and DGP. This is not surprising since P intakes were in general higher than requirements (see *Table 2*). Similarly, the class of dietary protein had no significant effects on productive and economic parameters. Class of starch influenced ADG, with better values for the high as respect to the low class (1.41 kg/d vs 1.35 kg/d), and DGP, with better values for intermediate class (2.55 €/d) and high class (2.50 €/d) as respect to low class (2.33 €/d).

CONCLUSIONS

The productive performances of intensive North-East Italy beef sector were not influenced by phosphorus content of diet. As a consequence, P content of most diets appeared in excess, and it could be reduced without impairing growth performances In relation with P environmental fate, and its impact on promoving eutrophication of surface waterbodies, this reduction can be an important tool to improve the relation between the local beef sector and the local environment.

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The effect of a second grazing period on carcass traits of indigenous Cika and Simmental young bulls

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ABSTRACT

Carcass quality of Cika (20) and Simmental (19) young bulls either semi-intensively fattened indoors or finished indoors after a previous (second) grazing period was studied. There was no difference between breeds for carcass weight, dressing percentage, EUROP conformation, carcass length as well as the proportion of fore-stomachs and head. Only, fatness score and pelvic/kidney fat percentage were higher in Cika bulls compared to Simmental, while chest depth was higher in Simmental bulls. Bulls which have been finished indoors after a previous grazing period had significantly lighter carcasses, lower dressing percentage, lower EUROP conformation and fatness score but higher empty reticulo-rumen percentage compared to bulls fattened indoors. Breed x fattening technology interaction was significant for carcass weight, conformation, fatness, chest depth and empty reticulo-rumen, while slaughter weight affected only carcass weight, conformation and carcass length. The difference in the lean meat percentage between Cika and Simmental bulls was not significant. Cika bulls expressed higher fat and lower bone percentage compared to Simmental bulls. However, fattening technology did not affect the tissue percentages in the carcasses. Cika young bulls had more red and more yellow beef compared to Simmental. Bulls fattened indoors had slightly darker beef than bulls finished indoors after a second grazing period. However, carcass traits of Cika bulls were similar to those of Simmental bulls and a second grazing period could be efficiently set up in the growing-fattening scheme.
(Keywords: Cika, Simmental, young bulls, second grazing period, carcass traits)

INTRODUCTION

Nowadays indigenous Cika cattle (*Simčič et al.*, 2013) is considered a low milk productivity breed compared to popular commercial breeds and is mainly reared in herds with cow-calf system, despite the fact that in the past it was used mainly for milk production. On the other hand, dual purpose Simmental breed is the most widespread breed in Slovenia. Grazing season of suckler herds starts in the spring and lasts until late autumn when calves are weaned. Female calves are used for herd maintenance and preservation of endangered breed in the case of Cika. Male weaned calves are even slaughtered or fattened indoors (*Žgur et al.*, 2013). Usually the young growing fattening bulls are maintained indoors but a grazing period could be set up in the growing-fattening scheme, e.g., a first grazing season as calves in the suckler herds, a first indoor period as young stock, a second grazing season starting at 300–350 kg and a final finishing period indoors (*Dieuguz Cameroni et al.*, 2006). Regarding natural conditions

in Slovenia where cattle diet is based on the forage, the second grazing period for bulls could be easily adopted to the previously mentioned technology. The aim of this study was to investigate the effect of a second grazing period on the carcass traits of Cika and Simmental young bulls.

MATERIAL AND METHODS

Animals

The study was performed *in nature* and included 39 young bulls. Twenty Cika and 19 Simmental young bulls were bought from farms throughout Slovenia in November 2010 and housed in a feedlot with a closed barn with multiple pens for the winter time (178 days in average). During this period, all bulls were fed the same extensive total mixed ration based on maize and grass silage with a limited amount of concentrates. The experimental period started on May 2011. The young bulls of both breeds were divided into two subgroups according to their live weight. The first subgroup consisted of 10 Cika (initial weight 445.7 kg) and 9 Simmental (initial weight 392.4 kg) bulls that were fattened indoors with semi-intensive total mixed ration (S-INT) consisted of maize silage (66.0%), grass silage (16.5%), corn (9.5%), sunflower meal (7.1%) and mineral - vitamin premix (0.9%) that is commonly used for bulls fattening. Bulls were housed in four pens with a fully slatted floor, equipped with two drinkers to allow *ad libitum* water accessibility. Second subgroup included 10 Cika (initial weight 339.7 kg) and 10 Simmental (initial weight 312.5 kg) bulls that were put on all-day grazing in the pasture, divided in three paddocks, with *ad libitum* water and minerals access. Grazing period finished in October 2011, at the end of the vegetation season and lasted in total 131 days. Cika bulls were lighter (427.7 kg) compared to Simmental (434.5 kg) at the end of second grazing period. After that, bulls were housed in four pens and finished with the same S-INT total mixed ratio that the first subgroup also received. All bulls were weighted at the beginning of the experimental period and thereafter, once a month until slaughter time. The average daily gain (ADG) was calculated dividing the difference between final and initial live weight by the number of days of the period. All the bulls were slaughtered when they achieved appropriate commercial finishing according to Slovenian market requirements.

Carcass quality

Young bulls fattened indoors were slaughtered at an average slaughter weight of 674.4 kg for Cika and 668.9 kg for Simmental, while young bulls finished indoors after a previous second grazing period at 606.4 kg and 663.6 kg, respectively. During the slaughtering process, head (without skin and horns), full and empty reticulo-rumen and full omasum/abomasum, pelvic/kidney fat were weighted. After slaughter, hot carcasses were weighed and dressing percentage was calculated as hot carcass weight divided by slaughter weight. Carcasses were graded for conformation (EUROP) and fatness according to the European grading scheme. Carcass length was measured as a distance from the front edge of the pelvic symphysis to the middle of the front edge of the first rib. Chest depth was measured as the distance from the ventral edge of the spinal canal to the ventral edge of the broken sternum of the fifth rib. pH 24 was measured 24 h *post mortem* in the middle of cross section of *Longisimus dorsi* muscle between 6^{th} and 7^{th} rib. Beef colour was measured as a triplicate on the same LD cross section after 30 minute of exposure to the air by chromo meter (Minolta CR 300) and expressed as CIE L*a*b* values. After chilling, the right carcass side was separated into the main carcass

tissues (lean meat, fat, tendons, and bones). The total weight of separated tissues was used to calculate the percentage of four various tissues in the carcass.

Statistical analysis

Data was analysed using the GLM procedure in the statistical package SAS/STAT (SAS Institute Inc., 2001). The effect of breed (B), fattening technology (T), breed by fattening technology interaction (B x T) and slaughter weight as linear regression were included in the model. For carcass tissue composition and meat colour the B x T interaction and slaughter weight as co-variable were omitted from the model as they were not significant.

RESULTS AND DISCUSSION

The second grazing period is commonly used in extensive beef production with steers, for example of Rubia Galega, a local beef breed from Spain (*Varela et al*., 2004), dairy Holstein and Montbéliard breeds in France (Thénard et al., 2006) as well as Charolais x Friesian crossbreds in Ireland (*Keane and Allen*, 1998). Some of them are finished in the pasture others are finished indoors after grazing period. However, there is lack of literature studied bulls fattening in the pasture as a consequence of not commonly used bulls as grazing animals. An exception is a report from *Piedrafita et al*. (2003) about Aubrac bulls, an indigenous breed from France. After weaning, the Aubrac bulls were reared indoors during first winter, then put on the pasture until 19 months of age and then were finished indoors until slaughter at 29.2 months of age. Likewise, *De la Fuente et al*. (2009) studied Fleckvieh x Limousine crossed bulls raised in the pasture and finished indoors.

Simčič et al. (2010) studied carcass quality of Cika bulls finished in the pasture. At the slaughter age of 23.5 months they achieved only 232.8 kg carcass weight, conformation score 5.2 and fatness score 3.4. Regarding poor carcass quality authors recommended to finished Cika bulls indoors after grazing period to achieve larger slaughter weight and better conformation and fatness scores.

In this study, indoor fattened Cika bulls had only slightly lower ADG during the whole fattening (817 g/day) compared to Simmental (837 g/day) young bulls. On the pasture, the difference in ADG between both breeds was more pronounced. During grazing period ADG of Cika bulls was much lower (662 g/day) compared to Simmental (917 g/day) bulls. This difference diminished after bulls were housed and fattened again indoors. Cika bulls achieved 842 g/day while Simmental bulls 909 g/day. This much lower ADG in Cika bulls could be at least partly explained by earlier sexual maturity of Cika bulls and consequently more aggressive and sexual behaviour during grazing.

Among all included carcass traits, there were no significant differences between breeds for carcass weight, dressing percentage, EUROP conformation, carcass length as well as the proportion of fore stomaches (full reticulo-rumen, empty reticulo-rumen, and full omasum/abomasum) and head proportion. Only fatness score and pelvic/kidney fat percentage were significantly higher in Cika (6.18±0.18, 1.08±0.06%) compared to Simmental (5.21±0.19, 0.70±0.06%) bulls, respectively, whereas chest depth was significantly higher in Simmental (45.57±0.27 cm) compared to Cika (44.38±0.26 cm) bulls (*Table 1*). Carcass weight, dressing percentage and conformation score of Simmental bulls were similar to those reported by *Albertí et al*. (2008) but, due to the low energy content of S-INT finishing total mixed ratio, the animals were older at slaughter and carcass had a lower fatness score than the reported one.

Bulls which were finished indoors after a previous grazing period had significantly lighter carcass (356.67±2.20 kg), lower dressing percentage (54.62±0.34%), lower EUROP conformation (8.51±0.17) and fatness score (5.34±0.18) and higher empty reticulo-rumen proportion (1.78±0.04%) compared to bulls fattened indoors with S-INT diet (368.89±2.26 kg, 56.50±0.34%, 9.11±0.17, 6.05±0.19), respectively (*Table 1*). The pH 24 was also slightly higher in bulls that were previously grazed before indoors finishing.

De la Fuente et al. (2009) investigated Fleckvieh x Limousine crossed bulls raised in the pasture and finished on corn silage *at libitum* supplemented with soy and cereal meal during last six months. Bulls were slaughtered at 19–24 months and had a little higher carcass weight (382.4±41.1 kg) compared to indoors fattened bulls in this study. On the other hand, Aubrac bulls finished indoors after second grazing period achieved higher (451.0 kg) carcass weight and higher dressing parcentage (59.9) at higher slaughter age 722.8 days (29.2 months) compared to Cika and Simmental bulls in similar rearing technology. Likewise, conformation (9.5) and fatness (7.8) were higher at Aubrac compared to bulls from this study finished indoors after grazing (8.51, 5.34), respectively (*Piedrafita et al.*, 2003).

Table 1

Carcass traits of Cika and Simmental young bulls from different fattening technologies (LSM±SE)

Carcass traits	Effects							
	Breed (B)			Fattening technology (T)			B x T	SW
	Cika	SIM	p-value	S-INT	Grazing + S-INT	p-value	p-value	p-value
Carcass weight (kg)	359.86 ±2.15	365.70 ±2.21	n.s.	368.89 ±2.26	356.67 ± 2.20	0.001	0.041	<0.001
Dressing percentage (%)	55.12 ±0.33	56.00 ± 0.37	n.s.	56.50 ±0.34	54.62 ±0.34	0.001	n.s.	n.s.
EUROP conformation* (score 1–15)	8.73 ±0.16	8.90 ± 0.17	n.s.	9.11 ±0.17	8.51 ±0.17	0.022	0.040	0.035
Fatness (score 1–15)	6.18 ±0.18	5.21 ± 0.19	0.001	6.05 ±0.19	5.34 ±0.18	0.015	0.042	n.s.
Carcass length (cm)	138.32 ±0.61	138.90 ±0.63	n.s.	138.61 ±0.65	138.60 ±0.63	n.s.	n.s.	<0.001
Chest depth (cm)	44.38 ±0.26	45.57 ±0.27	0.001	45.01 ±0.28	44.95 ± 0.27	n.s.	0.043	n.s.
Pelvic/kidney fat (% SW)	1.08 ±0.06	0.70 ±0.06	<0.001	0.87 ±0.06	0.91 ±0.06	n.s.	n.s.	n.s.
Full reticulo-rumen (% SW)	9.18 ±0.34	9.05 ±0.35	n.s.	8.72 ±0.35	9.50 ±0.34	n.s.	n.s.	n.s.
Empty reticulo-rumen (% SW)	1.72 ±0.04	1.66 ±0.04	n.s.	1.60 ±0.04	1.78 ±0.04	0.006	0.041	n.s.
Full omasum/ abomasum (% SW)	2.63 ±0.07	2.63 ±0.07	n.s.	2.54 ±0.08	2.72 ±0.07	n.s.	n.s.	n.s.
Head (% SW)	4.38 ± 0.07	4.53 ± 0.07	n.s.	4.44 ± 0.07	4.48 ± 0.07	n.s.	n.s.	n.s.
pH 24	5.62 0.01±	5.61 ±0.01	n.s.	5.59 ±0.01	5.64 ±0.01	0.048	0.015	n.s.

SIM–Simmental; SW–slaughter weight; S-INT–semi intensively total mixed ratio; n.s.–p>0.05; *(E+ = 15, E◦ = 14, E- = 13, U+ = 12, U◦ = 11, U- = 10, R+ = 9, R◦ = 8, R- = 7, O+ = 6, O◦ = 5, O- = 4, P+ = 3, P◦ = 2, P- = 1)

Moreover, breed x fattening technology interaction in this study was significant for carcass weight, conformation, fatness, chest depth, empty reticulo-rumen and pH 24, while slaughter weight significantly affected only carcass weight, conformation and carcass length (*Table 1*).

In the carcasses, lean meat represented the largest percentage, but the difference between Cika (72.29±0.41%) and Simmental bulls (73.14±0.42) was not significant. Breed significantly affected fat and bone percentages. Carcass of Cika bulls contained more fat (11.60±0.32%) and fewer bones (14.48±0.22%) compared to Simmental bulls (8.79±0.33%, 16.31±0.22%). Fattening technology did not affect the percentage of the tissues in the carcasses (*Table 2*). Similar percentages of tissue composition estimated from the sixth rib (76.1% lean meat, 15.4% bones, 7.6% fat) found *Piedrafita et al.* (2003) in carcasses of Aubrac bulls finished indoors after grazing period.

Cika young bulls had more red (a*) and more yellow (b*) meat (27.03±0.52; 12.23±0.28) compared to Simmental (24.75±0.53; 11.02±0.28). However, bulls fattened indoors had slightly darker meat (35.51±0.36) than bulls finished indoors after a second grazing period (36.76±0.35) (*Table 2*).

Table 2

Proportions of main tissues in the carcass and meat colour of Cika and Simmental young bulls from different fattening technologies (LSM±SE)

	Effects					
	Breed (B)			**Fattening technology (T)**		
	Cika	**SIM**	**p-value**	**S-INT**	**Grazing + S-INT**	**p-value**
Lean meat (%)	72.29 ± 0.41	73.14 ± 0.42	n.s.	72.97 ± 0.42	72.45 ± 0.41	n.s.
Fat (%)	11.60 ± 0.32	8.79 ± 0.33	<0.001	10.02 ± 0.33	10.37 ± 0.32	n.s.
Tendons (%)	1.64 ± 0.04	1.76 ± 0.04	n.s.	1.69 ± 0.04	1.70 ± 0.04	n.s.
Bones (%)	14.48 ± 0.22	16.31 ± 0.22	<0.001	15.32 ± 0.22	15.47 ± 0.22	n.s.
L* value	36.15 ± 0.35	36.12 ± 0.36	n.s.	35.51 ± 0.36	36.76 ± 0.35	0.018
a* value	27.03 ± 0.52	24.75 ± 0.53	0.004	25.47 ± 0.53	26.31 ± 0.52	n.s.
b* value	12.23 ± 0.28	11.02 ± 0.28	0.004	11.35 ± 0.28	11.90 ± 0.28	n.s.

SIM–Simmental; S-INT–semi intensively total mixed ratio; n.s.–p>0.05

CONCLUSIONS

Carcass traits of Cika bulls were similar to those of Simmental bulls and should encourage breeders to fatten young Cika bulls for beef production and contribute to the maintenance of endangered indigenous Cika breed. A second grazing period could be efficiently set up in the growing-fattening scheme as it would not significantly decrease carcass traits except dressing percentage. Moreover, an improvement of growth performance could be achieved by increasing the energy level of the finishing diet, particularly for the Simmental bulls.

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Survey on mortality rate of young stock on dairy farms of the Province of Padova

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ABSTRACT

The present study aimed at investigating preliminarily the mortality rate in 2012 of replacement calves and heifers in 95 dairy farms in the Province of Padova. Data regarding total number of cows and replacement stock reared were gathered from the APA Breeders' Association records while data on number of dead replacement animals were collected from insurance records. Results showed that the median value of the overall mortality rate of replacement cattle <24 months was 3.3% (0–6.7%, first - third quartile) with a maximum of 28.6%. Considering the age categories, mortality of 0–12 months old replacement cattle was higher than that of 13 - 24 months old. The median of mortality for cattle <12 months was 4.9% (0–11.8%, first - third quartile), with a maximum of 72.1% in one farm. The median value of mortality for the older age category (13–24 months) was of 0% (0–1.6%, first - third quartile) with a maximum value of 25%. This seems a positive outcome, however, the high variability arisen from this survey points out serious problems in some farms. Moreover, despite results pointed out a higher risk of mortality at the early stage of cattle life, a considerable number of farms showed mortality rates exceeding 10% for the heifers between 13 and 24 months of age. Mortality rates at a late stage indicate a serious situation in these farms which leads also to relevant economic losses. In conclusion, it is suggested to investigate on predisposing risk factors at different ages in order to develop and apply specific actions to overcome such problems.

(Keywords: calf, replacement dairy cattle, mortality)

INTRODUCTION

Italian intensive dairy farms are generally characterized by a good management for lactating cows, but little attention is paid to calves and heifers with a negative effect on their welfare. Since the farm's profit depends on the incomes from milk, the improvement in cows welfare conditions have a direct and tangible economic return. Replacement heifers start to generate profit only after the first calving, so the economic losses related to poor management of these animals until that moment are rarely quantified. Due to a culling rate of around 30%, assessed for the Italian Holstein cows, at least half of the animals raised on each dairy farm is represented by young stock and replacement costs can, therefore, reach 15–25% of the total cost for milk production (*Mourits et al.,* 1999). Factors that can affect the replacement rearing costs are culling rate, age at the first calving, mortality and age at death of young stock. Among these

factors, the mortality of young stock is very often underestimated by farmers. A recent study by *Mee* (2013) reported that farmers can underestimate the incidence of calf diseases by up to 40%, and loss rates till 50% and found a very low correlation (r = 0.01) between actual and perceived mortality rates. Farmers often don't apply the recommendations that they receive from veterinarians and technicians, mostly because they don't perceive the problem and they still consider calves as by-product of milk production and not as "the cow of the future". The result is that despite the modernization of intensive dairy farms, young stock losses are still rising in many European countries (*Mee,* 2013). The present study investigated the mortality rate of replacement calves and heifers in 95 dairy farms in the Province of Padova with the aim to lay the groundwork for future investigations on the main critical points from birth to first calving which can cause losses of young stock.

MATERIAL AND METHODS

Ninety-five Italian Holstein dairy farms belonging to the Provincial Breeders Association (APA) and located in the Province of Padova were considered in the study. All selected farms subscribed an insurance which refunds farmers for the loss of the animal and its carcass disposal costs. For each farm, data referred to year 2012 were gathered by investigating the APA and the insurance records. Data related to total number of cows and number of young stock considering the category from 0 to 12 months and from 13 to 24 months were gathered from the APA records. Number of animals dead in each of the four age categories: 0–2, 3–6, 7–12, and 13–24 months were collected from the insurance records.

Data processing and statistical analysis

Overall replacement mortality of cattle < 24 months of age was calculated as a ratio between number of dead animals and total number of alive young cattle (0–24 months). Mortality rates were calculated using the same approach also for the two age categories (from 0 to 12 and from 13 to 24 months).

The percentage of animals dead in each age category (0–2, 3–6, 7–12, 13–24 months) was calculated over the total number of young animals dead in order to highlight potential risks due to age.

All data were first submitted to descriptive statistics to assess location parameters. Mortality rates were the independent variables whereas farm size (≤50, 51–100, >100 dairy cows reared) was the dependent variable.

RESULTS AND DISCUSSION

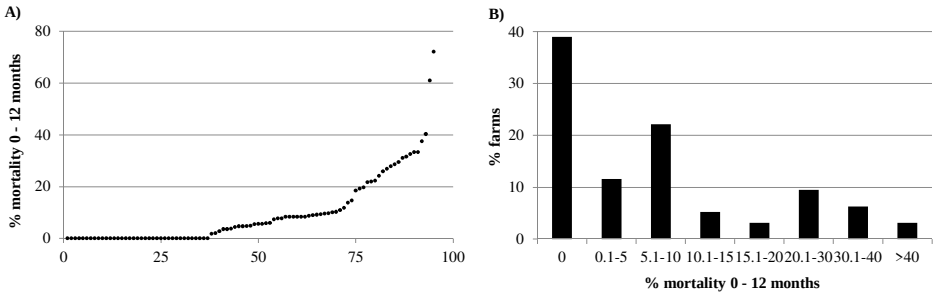
The farms included in the study showed a wide range of herd size from a minimum of 15 to a maximum of 523 cows. The average number of cows reared in 2012 was 92.6±78.7 (±SD). The class of small size (≤50 cows) included 26 farms, 39 farms were medium (51–100), and 30 farms were large (>100 cows).

Overall mortality rate of replacement cattle <24 months was not normally distributed and the median value was 3.3% (0–6.7%, first - third quartile). This seems a positive outcome considering that the Dairy Calf and Heifers Association Gold Standards (*DCHA Gold Standards*, 2013) suggests that the cumulative mortality rate should not exceed 10% for calves and heifers from one day of life to the first calving. However, the high

variability arisen from this survey points out serious problems in some farms. The loss rates are even more alarming if considering the single age categories (*Figures 1 and 2*). Distribution of mortality of the younger age category (0–12 months) in the 95 farms showed a median of 4.9% (0–11.8%, first - third quartile), with a maximum of 72.1% in one farm (*Figure 1A*). Twenty-seven percent of the farms (*Figure 1B*) had a mortality rate higher than the threshold value of acceptability (*DCHA Gold Standards,* 2013), and in these farms in particular, predisposing risk factors should be investigated and specific actions should taken in order to overcome such problems.

Figure 1

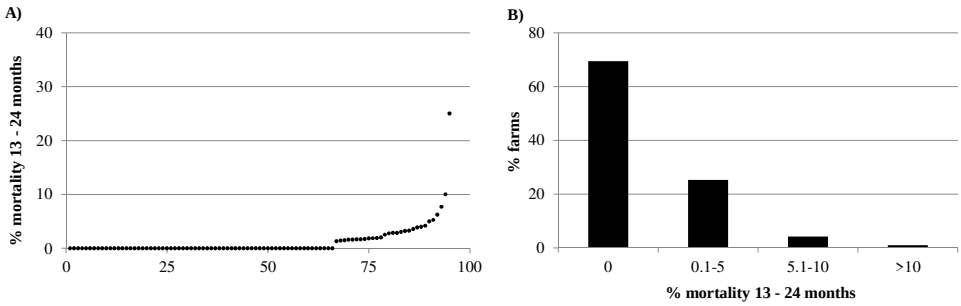
Mortality of replacement cattle between 0 and 12 months: distribution of mortality rates of the 95 farms (A) and distribution of farms according to classes of mortality (B)



As expected from a previous study (*Svensson et al.*, 2006), the mortality rate was lower for the older age category (13–24 months), with a median value of 0% (0–1.6%, first - third quartile), and a maximum value of 25% (*Figure 2A*). However, over 70% of farms had mortality rates above the 0.5% threshold value acceptable for replacement cattle older than 12 months until freshening (*DCHA Gold Standards,* 2013).

Figure 2

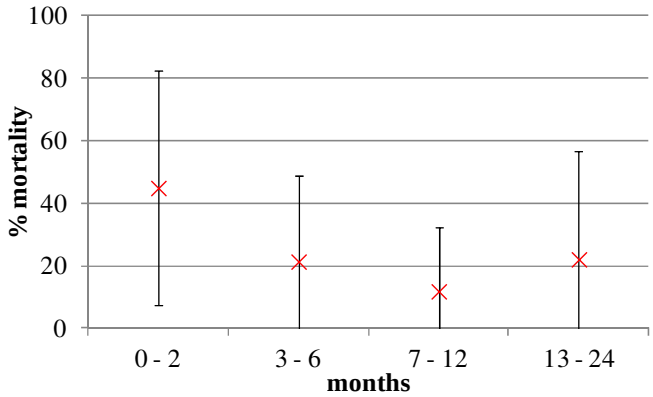
Mortality of replacement cattle between 13 and 24 months: distribution of mortality rates of the 95 farms (A) and distribution of farms according to classes of mortality (B)



Analysing into more details the percentage of animals dead in each of the four age categories (0–2, 3–6, 7–12 and 13–24 months) among the dead replacement cattle, it must be pointed out that the higher mortality rate was observed for calves between 0 and 2 months (*Figure 3*). This finding is in accordance with the results of previous studies carried out on replacement cattle either in the USA (*Wells et al.,* 1996; *Sivula et al.,* 1996), in Sweden (*Svensson et al.,* 2006) and in Italy (*Colnago et al.,* 2007). A plausible explanation to the higher mortality of calves in the first months of life is their susceptibility to diseases, especially enteric and respiratory, due to a poorly competent immune system (*Sivula et al.,* 1996; *Wells et al.,* 1996). Moreover, the stressful conditions such as calves' separation from the dam, and changes in housing (individual *vs.* group) and diet (weaning) might act as important predisposing factors to disease outbreak and consequently to mortality (*Wells et al.,* 1996; *Stull and Reynolds*, 2008; *Zucali et al.,* 2013). In accordance with results by *Svensson et al.* (2006), mortality in the current study tended to decrease progressively from the third month to a year of life and to increase again after this age interval (*Figure 3*). In the current study, indeed, the average mortality rate reached 22% for 13–24 months old heifers. Causes of mortality for this age category could not be the same of those acting at an early stage but should be identified among housing facilities or management. Trauma as consequence of overcrowding, hierarchy establishment and inappropriate flooring, and peripartum disorders are main predisposing factors for mortality at this age (*Bøe and Færevik,* 2003; *Svensson et al.*, 2006; *Dorigo et al.,* 2009).

Figure 3

Distribution of mortality (average percentage±SD) at different ages over the total number of replacement cattle dead in the 95 farms



Regardless of farm size that did not affect mortality rates (P>0.05) and age category in which mortality occurs, the high variability among farms, makes it necessary to differentiate good and bad performing farms. In order to identify the best and the worst situation, farms were distributed on the basis of the mortality rates of the two age categories (0–12 and 13–24 months) within the mortality thresholds defined by the *DCHA Gold Standards* (2013). Twenty-six farms (27.4%) could be considered the best since they had mortality rates lower than 1% for all young cattle (*Table 1*). Thirty-four farms (35.9%) could be considered as well performing since they fell in the acceptable

range of mortality below 10% for the calves (0–12 months) and below 1% for the heifers (13–24 months). None of the farms had mortality rates over 10% for both age categories indicative of the worst possible situation. However, a considerable percentage of farms (28.4%) showed low or acceptable rates of mortality for the age category between 0 and 12 months, but mortality rates exceeding 10% for the heifers between 13 and 24 months of age. Such mortality rates at a late stage indicate a serious situation in these farms which leads to relevant economic losses. The latter are proportional to the age at death due to the incurred rearing costs and the acquisition of new replacement heifers (*Campiotti,* 2012). Regardless of the age category in which mortality occurs, the loss of replacement cattle is not only an economic problem but also a health problem, since the introduction of new heifers by external dairy farms increase the risk of introducing new diseases.

Table 1

Distribution of the dairy farms (%) on the basis of replacement cattle mortality rates of the two age categories (0 - 12 and 13 -24 months) within the mortality thresholds defined by the *DCHA Gold Standards* (2013)

		Mortality 0–12 months			
		<1%	1–5%	5.1–10%	>10%
Mortality 13–24 months	<1%	27.4	7.4	1.1	1.1
	1–5%	6.3	6.3		
	5.1–10%	15.8	4.2	2.1	
	>10%	18.9	7.4	2.1	

CONCLUSIONS

The results of this preliminary investigation indicated that management of young cattle is still a critical point in a large number of dairy farms considering the high variability of the mortality rates. Although results pointed out a higher risk of mortality at the early stage of cattle life, a considerable number of farms showed mortality rates exceeding 10% for the heifers between 13 and 24 months of age. Mortality rates at a late stage indicate a particularly serious situation in these farms which leads also to relevant economic losses.

In conclusion, it is suggested to investigate on predisposing risk factors at different ages in order to develop and apply specific actions to overcome such problems.

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Carbon footprint from dairy farming system: comparison between Holstein and Jersey cattle in Italian circumstances

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ABSTRACT

Aim of the present study was to estimate the carbon footprint (CF) of milk production at farm gate considering two dairy cattle breeds, Holstein Friesian (HF) and Jersey (JE). Using Italian inventory data the emissions of CO_2eq per kg ECM for dairy herds of HF and JE breed were estimated. The results show 0.80 kg CO_2eq/kg ECM in JE herd, while 0.96 kg CO_2eq/kg ECM in HF herd. The main differences were due to the level of dry matter intake, milk yield and fertility traits. Indeed, JE herd showed a lower milk yield than HF herd, a lower DMI and better fertility, determining less production and consumption of feed and less replacement animals in the herd.
(Keywords: carbon footprint, dairy cattle breed, milk production)

INTRODUCTION

Carbon footprint (CF) is the total amount of GHG emitted in production processes, expressing Global Warming Potential (GWP). According to *IPCC* (2006), GHG attributed to the agricultural activity are methane (CH_4) and nitrous oxide (N_2O). CH_4 is produced mainly with enteric fermentation (*Cassandro et al.,* 2013) and decomposition of manure, while N_2O derives from the N content of manure and from N of fertilizers once they are applied to the soil.

The major part of studies about CF on dairy milk production are lacking on variation of CF across different dairy breed. Indeed, *Cassandro* (2013) compared local and cosmopolitan cattle breeds on their predicted methane emissions showing that a reduction of 10% of daily methane emissions per kg of metabolic body weight is expected for local compared with cosmopolitan breeds. Moreover, *Capper and Cady* (2012) published CF results from comparison between Jersey (JE) and Holstein Friesian (HF) cattle breeds, where production of the same quantity of protein, milk-fat, and other solids, Jersey cows emitted 20% less CF.

Aim of this study was to investigate the difference of CF among HF and JE dairy herd in Italian circumstances, using an holistic approach.

MATERIAL AND METHODS

A life cycle assessment (LCA) in a farm gate perspective was performed to evaluate CF of HF and JE dairy breed in Italy. Using data from national inventories a standard dairy herd was performed and a linear model was developed, using *Excel Software (Microsoft,* 2010), and it were estimated and assumed inputs, outputs, herd turnover and GHG emissions. Reference time was one year.

The system included: (i) GHG emissions (defined as CO_2eq) derived from the production of one kg of dry matter of feed, and straw as bedding materials, used in the herd; (ii) emissions of CH_4 derived from enteric fermentation; (iii) emissions of CH_4 and N_2O (direct and indirect) associated with manure management. Emissions from other production inputs like pesticides, seeds, fossil energy consumption, liming and medicine were not included as well as the information of the construction of machinery and buildings or the potential emission from managed organic soil (*Kristensen et al.,* 2011).
One kg ECM (*Sjaunja et al.,* 1990) produced at farm gate was chosen as functional unit.
Biological allocation was applied, where allocation factors to milk and meat were calculated (AFmi and AFme, respectively), which are used to share GHG emissions between the amount of milk and meat according the energy required to produce the two outputs (Live Weight, LW) (*IDF,* 2010). The organization of herd system took into account a typical intensive farming system used in Northern Italy.

Two animal systems composed the herd: (i) cow (including dry and lactating dairy cows), (ii) heifer (including heifers destined to replacement, and exceed heifers used to fattening). Moreover (iii) calf system (male calves destined to fattening), reared as veal calves, a typical production of in the Italian cattle livestock (*Dall'Orto et al.,* 2010), was considered in HF herd. Calf system in JE herd was not considered because we assumed that they leave the herd system immediately after birth (*Capper and Cady,* 2012).
One hundred cows were the basis for the calculation of herd turnover. Two numbers of animals were estimated for herd turnover: animals annually feed (sum of feeding days/365days) and animals annually slaughtered. Both numbers were computed considering number of animals born in one year and the months spent inside the herd by each animal system. For heifer system this value was the months at first calving while in male calf system it was months at slaughtering.

Animals annually feed were calculated considering several parameters: calving interval, replacement rate, stillbirth rate and female rate. Artificial insemination was the only reproduction technique (no bulls were present). Animals annually slaughtering were computed after considering the mortality rate. LW (kg) obtained in the herd was calculated considering the animal weight before slaughtering for each of animal system. Heifers were assumed to be replacement animals, from birth to first calving. Surplus heifers, which exceed the replacement rate, were assumed to be slaughtered at the normal age of first calving. Buying and selling of animals were not taken into account.

Feed ration was calculated for each animal system. Daily dry matter intake (DMI), content of crude protein (CP), ash (Ash), daily gross energy (GE) assumption and digestibility of organic matter (DE) were identified. The feed ration for JE cow and heifer system was obtained using proportional data derived from the average LW ratio of the two breeds in the respective animal system. The feed rations were calculated using literature review (not show in this paper).

Information about stable system was modeled for cow and heifer (*CRPA,* 2012) and calf (*Mottaran*, 2011; *Dell'Orto*, 2010) system using literature data. The GWP was estimated for a 100-year time period by converting all GHG to CO_2 equivalents

(CO2eq), which on a weight basis gives 1 kg CH_4=25 and 1 kg N_2O-N=298 CO_2eq (*IPCC,* 2006). The GHG emissions, expressed as kg CO_2eq, were determined per herd, per kg ECM and kg meat (LW). The emission factor (EF) to CH_4 enteric emissions was calculated using equation for dairy cows by *Ellis et al.* (2007) (CH_4 (MJ/d) = 3.23 (± 1.12) + 0.809 (± 0.0862) × DMI (kg/d)) and considering an energy content of 55.65 MJ in 1 kg of CH_4 (*IPCC,* 2006). CH_4 and N_2O emissions from deep litter and slurry manure produced by herd was estimated using the IPCC Tier 2 method *IPCC* (2006) using specific country parameters (*INIR,* 2012), but international values (*IPCC,* 2006) were used in some cases, according the Italian national emissions inventory (*INIR,* 2012).
N excretion rate were derived from N intake, subtracting the N contained in milk and meat produced, and N in the bedding straw (*Kristensen et al.,* 2011). Emissions CO_2eq per kg dry matter of feed were derived from literature (*Guerci,* 2012).

RESULTS AND DISCUSSION

HF herd emitted 1,188,321 kg CO_2eq, while JE herd were 39% lower than HF herd. The main source of GHG was Total CH_4, which represented 59% and 63% of CF, for HF and JE herd, respectively. Enteric CH_4 represented 75% and 78% of Total CH_4 emissions. CF deriving from production and utilization of feed was the second source of GHG representing 37% in HF herd and 30% in JE herd of total GHG emissions. Thirdly emitter was N_2O emissions, 7.5% of the total GHG emissions in both herds.

Cow system was the first emitter of GHG in the herd, emitting 62% and 68% in HF and JE herd, respectively. Second emitter was heifer system, releasing 28% and 32% in HF and JE herd, respectively. While calf system, only present in HF herd, emitted 10% of total emissions. Milk production had the greatest part of the emissions in both herd system *(Table 1)*, recording 72% and 80%, as AFmi, of total GHG emissions, for HF and JE herd, respectively.

Emission of CO_2eq, associated to ECM production was greater for HF herd (0.96 kgCO_2eq/kg ECM) than JE herd, which had 17% less than HF herd (0.80 kgCO_2eq/kg ECM). Similar lower trend in JE herd (23% less than HF herd system) was recorded for kgCO_2eq/kg meat. The main differences are number of heads, milk production and level of DMI in the herd among the two breeds considered.

HF herd presented higher calving interval (HF: 432 days; JE: 385 days), replacement rate (HF: 34%; JE: 30%) and age at first calving (HF: 28.4 months; JE: 26.0 months) than JE herd, which increased heads in the herd (HF: 218; JE: 197), replacement heifers (HF: 81; JE: 65) and culled cows (HF: 32; JE: 29); having more heads, higher emissions are produces, obviously. This shows a general better fertility of JE breed than HF breed and according *Garnsworthy et al.* (2004) a better fertility traits in the herd determine a lower GHG emissions from herd. Moreover HF herd presented calf system, which increase meat produced but at the same time the emissions. Removing calf system, the emissions from HF herd are 0.94 kgCO_2eq/kg ECM and 14.44 kgCO_2eq/kg meat, remaining higher than JE herd values.

HF herd had a greater milk yield (8,853 kg ECM/cow/year) than JE herd (7,239 kg ECM/cow/year), while JE herd presented higher values of fat and protein (fat: 4.98%; protein: 4.01%) respect HF herd (fat: 3.73%; protein: 3.39%). *Capper and Cady* (2012) published CF results of the comparison between Jersey and Holstein breeds; they found that for the production of the same quantity of protein, milk-fat, and other solids, Jersey cows emitted 20% less CF. If HF milk yield is decreased to same amount of JE herd the emissions increase to 1.07 kg CO_2eq/kg ECM, and if JE herd system produce same

amount of HF herd, the emissions per kg ECM decrease to 0.68 kg CO_2eq. According to *Capper and Cady* (2012), body weight, milk yield, and milk nutrient density differences between HF and JE breed have the greatest effect upon CF per unit of product. Level of feed intake and its composition are important factors influencing GHG losses (*Bell et al.,* 2012). JE breed, being a lightweight compared to HF breed (LW cow: 454kg and 700kg, respectively), and its DMI is lower of HF breed (DMI herd: 3,381 kg/year and 4,805 kg/year, respectively; where JE cow and heifer system consumed 65% of DMI of the respective HF animal system), corresponding to a lower GHG emissions, as noted by *Ferris* (2011).

The main impact category is represented by CH_4 from enteric fermentation, followed by emissions associated to feed production and thirdly CH_4 and N_2O emissions from manure. *Rotz et al.* (2010) determined as enteric CH_4 has the greatest effect on the overall CF, which principally depends upon milk production level and the feeding. The main differences between herd systems were level of DMI and milk yield, recognized from *Yan et al*. (2010) as the main drivers of enteric CH_4 emission.

Table 1

Emissions per head (kg CO_2eq/head), per animal system(kg CO_2eq/heads), per herd (kg CO_2eq/herd), per kg ECM and kg meat(kg CO_2eq/kg ECM and kg meat) and allocation factor (AF, %) for Italian Holstein Friesian (HF) and Jersey (JE) herd. Data concerning one year

	HF				JE			
	Cow	**Heifer**	**Calf**	**Herd**	**Cow**	**Heifer**	**Calf**	**Herd**
Feed[1]	2,197	1,120	5,382	1,984	1,425	727	0	1,080
Bedding straw[2]	0	27	35	15	0	21	0	10
CH_4								
Enteric CH_4	3,315	1,509	795	2,276	2,336	1,165	0	1,758
Manure CH_4	1,340	320	114	770	869	212	0	545
Total CH_4[a]	4,656	1,828	908	3,046	3,206	1,377	0	2,303
N_2O								
Direct N_2O	74	231	95	147	45	161	0	102
Indirect N_2O	443	127	11	262	267	79	0	174
Total N_2O[b]	517	358	106	409	312	240	0	276
Tot GHG/head[3]	7,370	3,333	6,431	5,455	4,942	2,364	0	3,670
Allocation Factor milk, %		72			80			
Allocation Factor meat, %		28			20			
kg CO_2eq/kg ECM		0.96			0.80			
kg CO_2eq/kg meat		15.43			11.88			

[1](kg CO_2eq/kg DM)*(kg DMI/head/year); [2] (kg CO_2eq/kg DM)*(kg straw/head/year).
[a]Kg CO_2eq derived from CH_4 emissions; [b] Kg CO_2eq derived from N_2O emissions.
[3]sum of emissions from Feed, Bedding straw, Total CH_4 and Total N_2O, per head of animal system in the herd (Cow, Heifer, Calf), and in the herd (Herd).

CONCLUSIONS

Asserting CF in milk production at farm gate several parameters affect the results: enteric CH_4 and CO_2eq from production and utilization of feed represent the main source of GHG emissions from dairy herd.

An important aspect to reduce the CF of milk production could be considered dairy cattle breeds inside the valuation. In this preliminary study JE herd system showed a lower CF per kg ECM than HF herd. Dairy cows were the first emitter in both herd. JE herd had lighter animals than HF breed, contributing a lower DMI in JE herd than HF herd. Moreover better fertility traits and higher values of fat and protein in milk was recognized in JE herd than HF herd. These parameters are the main contributors to lower CF in JE herd than HF herd.

As conclusions, a LCA could be applied to compare two dairy cattle herd, and other researches are suggested to show the deeply difference between the two dairy breeds and also including the followed staged of cheese production, an important Italian agricultural sector.

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Effect of breed and dairy system on milk composition and udder health traits in multi-breed dairy herds

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ABSTRACT

The aim of the present study was to investigate the effect of breed and dairy system on milk composition and udder health (UH) traits in multi-breed dairy herds. Individual milk samples (n=1,516) were collected from 41 multi-breed herds located in Trento province (in the North-East Italian Alps). Six breeds were involved: Brown Swiss (n=661), Holstein Friesian (n=473), Jersey (n=45), Simmental (n=158), Grey Alpine (n=75) and Rendena (n=104). Four different farming systems were identified: "Original Traditional" (lactating cows that are moved to highland pastures during summer; n=9), "Traditional without summer pastures" (n=11) "Traditional with silages" (n=2), and "Modern" (n=19). Analysis of variance was performed on milk composition and UH traits using the MIXED procedure of SAS. Orthogonal contrasts were estimated between least squares means of traits for the breed and the dairy system effects. Relevant differences have been highlighted between the six breeds regarding the milk composition traits, while breed differences for UH traits were negligible. The dairy system management revealed a limited influence on all considered traits.
(Keywords: milk composition traits, udder health traits, breed, dairy system, multi-breed herd)

INTRODUCTION

Increased milk production is one of the main dairy breeding goals worldwide dominating selection the last decades (*Meredith et al*., 2012). However, new breeding goals have recently been identified, especially on milk composition, following the demands of a healthier human diet (*Boichard et al*., 2012). Fat, protein and casein content are important traits for the milk and cheese industry while the fraction of milk used for cheese making is growing worldwide (*International Dairy Federation*, 2012). Milk urea nitrogen (MUN) is another interesting trait with remarkable environmental implications. Milk urea is synthesized as consequence of an imbalance between dietary nitrogen and energy in the rumen, and reflects inefficient protein synthesis. As the main non-protein source of nitrogen in milk, MUN reflects the efficiency of nitrogen utilization and the output of nitrogen to the environment. Among the functional traits**,** it is well known that udder health (UH) influence the qualitative and technological properties of milk. Somatic cell count (SCC) is commonly used as indicator trait of UH. Nevertheless, it has been recently reported that other traits such as lactose, pH, lactoferrin and minerals might be used as UH indicators (*Macciotta et al*., 2012). Lactose concentration decreases during mastitis and its association with SCC has been widely studied (*Kitchen*, 1981). In addition, it has been found that mastitis markedly influences the ionic environment in

milk. As a consequence of blood components moving into the milk, the pH may increase during mastitis (*Kitchen*, 1981). Finally, lactoferrin (an iron-binding glycoprotein which plays a key role as chemical barrier in defense mechanisms) concentration in milk is significantly associated with SCC (*Harmon et al*., 1975). An important question, though, before applying the new knowledge into practice, is to check whether there is a considerably source of variation of the aforementioned traits. To our knowledge, there are no studies on these traits in different breeds reared in multi-breed herds with different dairy system managements. Therefore, the aim of the present study was to investigate the effect of breed and dairy system on milk composition and UH traits in multi-breed dairy herds.

MATERIAL AND METHODS

Individual milk samples (n=1,516) were collected from 41 multi-breed herds located in Trentino region, Northern Italy, between March and December 2013. Six breeds were considered: Brown Swiss (BS, n=661), Holstein Friesian (HF, n=473), Jersey (Jer, n=45), Simmental (Si, n=158), Grey Alpine (GA, n=75) and Rendena (Ren, n=104). Herds were classified following the classification of Sturaro et al. (2013). Basically, four different farming systems were identified: "Original Traditional" (Orig-trad: lactating cows that are moved to highland pastures during summer; n=9), "Traditional without summer pastures" (Tr-nopast; n=11) "Traditional with silages" (Tr-si; n=2), and "Modern" (Mod; n=19). After collection, milk samples were refrigerated (4 °C) and processed within 24 hours from the collection. In the laboratory of the University of Trento individual milk subsamples were analyzed for protein, casein (%) and urea (mg/100 g) using a Milkoscan FT6000 (Foss, Hillerød, Denmark). In the laboratory of the University of Padova milk subsamples were analyzed for fat and lactose (%) using a MilkoScan FT2 (Foss, Hillerød, Denmark). SCC was obtained from a Fossomatic Minor (Foss, Hillerød, Denmark) and log-transformed to somatic cell score (SCS). Milk pH was obtained using a Crison Basic 25 electrode (Crison Instruments SA, Barcelona, Spain). Lactoferrin content (%) was measured by HPLC (High Performance Liquid Chromatography) analysis following the method of *Maurmayr et al*. (2013).

Analysis of variance was performed on milk composition and UH traits using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with the following linear model:

$$y_{ijklmn} = \mu + DIM_i + Parity_j + Breed_k + Dairy\ system_l + Herd_{m}\ (dairy\ system)_l + e_{ijklmn},$$

where y_{ijklmn} is the dependent variable; μ is the overall mean; DIM_i is the fixed effect of the *i*th class of days in milk (*i*=11 classes of 30-d intervals, from 5 to >305 d); $Parity_j$ is the fixed effect of the *j*th parity (*j*=1 to ≥6); $Breed_k$ is the fixed effect of the *k*th breed (*k*=BS, HF, Jer, Si, GA and Ren); Dairy $system_l$ is the fixed effect of the *l*th class of dairy system (*l*=Orig-trad, Tr-nopast, Tr-si and Mod); $Herd_{m}\ (dairy\ system)_l$ is the random effect of the *m*th herd (*m*=1 to 41) within the *l*th class of dairy system; e_{ijklmn} is the random residual ~ N (0, σ_e^2). Orthogonal contrasts were estimated between least-squares means (LSMs) of traits for the breed effect: a) specialized (BS, HF and Jer) vs dual purpose breeds (Si, GA and Ren); within specialized, b) BS+HF vs Jer and c) BS vs HF; within dual purpose, d) Si vs GA+Ren and e) GA vs Ren. Orthogonal contrasts were estimated also between LSMs of traits for the dairy system effect: a) traditional (Orig-trad, Tr-nopast and Tr-si) vs modern management; within traditional herds, b) Orig-trad+Tr-nopast vs Tr-si and c) Orig-trad vs Tr-nopast.

RESULTS AND DISCUSSION

Descriptive statistics for milk production, composition and UH traits are shown in *Table 1*. All traits exhibited high variability basically attributable to the breed differences. The results from ANOVA for the aforementioned traits are reported in *Table 2*. DIM and parity effects were important source of variation (P<0.001) for all the investigated traits, except for a negligible effect of parity on fat. In this study, particular interest was attributed to the breed effect, which is important in explaining the variability for all the analyzed traits, in particular for all milk composition traits and, within UH traits, for the lactose (P<0.001).

Table 1

Descriptive statistics of single test-day milk yield, composition and udder health traits[1]

Trait[2]	N	Mean	SD	P 1	P 99
Milk yield, kg/d	1451	24.32	9.15	6.00	49.40
Milk composition					
Fat, %	1495	4.22	0.92	1.88	7.12
Protein, %	1221	3.61	0.47	2.66	4.82
Casein, %	1224	2.84	0.38	2.10	3.80
Casein number	1224	0.78	0.01	0.75	0.81
MUN, mg/100 g	1224	25.0	9.6	7.5	49.0
Udder health					
Lactose, %	1510	4.97	0.29	4.10	5.52
pH	1510	6.51	0.10	6.27	6.74
SCC (10^3/mL)	1509	221	397	9	1,968
SCS, U	1509	2.84	1.86	-0.47	7.30
Lactoferrin, g/L	1492	0.097	0.052	0.026	0.236

[1] P1 = 1st percentile; P99 = 99th percentile
[2] MUN = milk urea nitrogen; SCS = log2 (SCC * 1,000/100,000) + 3

Our findings confirmed the results reported by *De Marchi et al*. (2007), who investigated differences in milk composition and coagulation traits in 5 dairy cattle breeds (BS, HF, Si, GA and Ren) sampled in mono-breed herds located in the same province considered for this study. In particular, excluding the Jer breed considered only in this paper, in both studies the milk of HF breed contained lower protein content (3.19% in *De Marchi et al.* (2007) vs 3.36% in our study), while the higher protein content was observed in BS (3.48% in *De Marchi et al*. (2007) vs 3.69% in our study).

Moreover, in the case of fat content, in both experiments a lower mean was observed in Ren (3.39% in *De Marchi et al*. (2007) vs 3.73% in our study) and a higher mean in Si (3.82% in De Marchi et al. (2007) vs 4.28% in our study). The effect of dairy system was negligible in explaining the variation of the former traits, except for protein and casein content (P<0.05) and for MUN (P<0.001). The highly significance of dairy

system for MUN was expected as urea synthesis is related to dietary nitrogen. For this trait, in fact, the proportion of variance explained by herd/test date was approximately 73% (*Table 2*).

Table 2

Results from ANOVA (F-value and significance) for single test-day milk yield, composition and udder health traits

Trait[3]	DIM	Parity	Breed	RMSE[1]	Dairy system	HTD, %[2]
Milk yield, kg/d	64.44***	28.19***	40.34***	5.05	2.48ns	54.75
Milk composition						
Fat, %	19.64***	1.06ns	33.3***	0.76	1.37ns	12.17
Protein, %	74.05***	6.2***	38.33***	0.30	3.43*	25.00
Casein, %	61.53***	7.71***	29.94***	0.25	3.31*	24.24
Casein number	8.35***	15.47***	4.32***	0.01	1.84ns	0.00
MUN, mg/100 g	3.88***	4.44***	7.90***	4.27	7.34***	73.05
Udder health						
Lactose, %	18.41***	20.64***	4.83***	0.25	1.07ns	13.79
pH	7.06***	9.29***	3.43**	0.07	1.84ns	0.00
SCS, U	17.68***	13.05***	3.47**	1.62	0.12ns	12.65
Lactoferrin, g/L	4.76***	4.27***	3.04**	0.05	1.52ns	0.00

[1]RMSE= root mean square error
[2]HTD, %= Herd/Test day effect expressed as proportion of variance explained by herd/test date calculated by dividing the corresponding variance component by the total variance.
[3]MUN = milk urea nitrogen; SCS = log2 (SCC * 1,000/100,000) + 3
ns= not significant; *P<0.05; **P<0.01; ***P<0.001

LSMs and orthogonal contrasts p-values of milk yield, composition and UH traits across breed and dairy system are reported in *Tables 3* and *4*, respectively.

Between the six breeds, HF displayed the highest milk yield (MY) (27.45 kg/d) and the lowest protein (3.36%) and casein (2.64%) content, while Jer shows the lowest MY (17.27 kg/d) and the highest fat (5.65%), protein (3.93%) and casein (3.10%) content. Specialized breeds reported higher fat, protein and casein percentages in comparison with the dual purpose breeds, while no significant differences was observed for MY, casein number and urea. Within the specialized breeds, there were relevant differences in almost all milk composition traits between BS and HF. With respect to MUN, a significant difference has been observed between BS and HF. We can assume that, on equal diet (BS and HF were mostly reared in the same multi-breed herds with a modern dairy system management), these two breeds have a different metabolism.

Table 3

Least squares means of single test-day milk yield, composition traits across breed and dairy system[1]

	MY, kg/d	Fat, %	Protein, %	Casein, %	Casein number	MUN, mg/100 g
Breed						
Brown Swiss (BS)	24.30	4.32	3.69	2.88	0.78	30.16
Holstein Friesian (HF)	27.45	4.04	3.36	2.64	0.78	27.91
Jersey (Jer)	17.27	5.65	3.93	3.10	0.79	28.44
Simmental (Si)	24.38	4.28	3.53	2.77	0.78	28.85
Grey Alpine (GA)	19.86	3.98	3.64	2.86	0.79	28.96
Rendena (Ren)	22.95	3.73	3.38	2.65	0.79	28.49
Contrast, p-value						
BS+HF+Jer vs Si+GA+Ren	0.255	<0.001	<0.001	<0.001	0.955	0.905
BS+HF vs Jer	<0.001	<0.001	<0.001	<0.001	0.004	0.651
BS vs HF	<0.001	<0.001	<0.001	<0.001	0.304	<0.001
Si vs GA+Ren	<0.001	<0.001	0.619	0.675	0.245	0.842
GA vs Ren	0.003	0.074	<0.001	<0.001	0.894	0.594
Dairy system						
Original traditional (Orig-trad)	18.37	4.28	3.57	2.79	0.78	34.56
Traditional no pasture (Tr-nopast)	21.47	4.15	3.38	2.67	0.79	27.83
Traditional with silages (Tr-si)	27.10	4.54	3.78	2.96	0.78	31.83
Modern (Mod)	23.86	4.36	3.62	2.85	0.79	20.98
Contrast, p-value						
Tr vs Mod	0.453	0.767	0.584	0.455	0.675	0.001
Orig-trad+Tr-nopast vs Tr-si	0.095	0.166	0.030	0.034	0.441	0.906
Orig-trad vs Tr-nopast	0.232	0.391	0.064	0.101	0.034	0.083

[1]MY = milk yield; MUN = milk urea nitrogen

HF, a breed selected for milk production, presented a lower level of MUN in the milk, in comparison with BS. Within the dual purpose breeds, Si, a large-sized widespread breed, reported higher MY and fat percentages in comparison with GA and Ren, small-sized local breeds. Considering the four dairy system classes, Tr-si showed the highest values of MY (27.10 kg/d), fat (4.54%), protein (3.78%) and casein (2.96%). The lowest value of urea synthesis has been found in modern dairy management (20.98 mg/100 g), while Orig-trad showed the highest value (34.56 mg/100 g). For protein, casein and casein

number only one contrast exhibited a significant p-value (Orig-trad+Tr-nopast vs Tr-si for protein and casein, Orig-trad vs Tr-nopast for casein number).

Table 4

Least squares means of udder health traits across breed and dairy system[1]

	Lactose, %	pH	SCS, U	Lactoferrin, g/L
Breed				
Brown Swiss (BS)	4.95	6.52	3.03	0.087
Holstein Friesian (HF)	4.96	6.52	3.23	0.097
Jersey (Jer)	4.83	6.51	2.85	0.093
Simmental (Si)	4.94	6.50	2.50	0.104
Grey Alpine (GA)	5.01	6.54	2.83	0.100
Rendena (Ren)	5.07	6.52	2.92	0.091
Contrast, p-value				
BS+HF+Jer vs Si+GA+Ren	<0.001	0.945	0.079	0.227
BS+HF vs Jer	0.005	0.334	0.349	0.969
BS vs HF	0.488	0.984	0.097	0.004
Si vs GA+Ren	0.003	0.004	0.084	0.189
GA vs Ren	0.182	0.068	0.760	0.332
Dairy system				
Original traditional (Orig-trad)	4.97	6.50	2.79	0.082
Traditional no pasture (Tr-nopast)	4.99	6.47	2.82	0.088
Traditional with silages (Tr-si)	4.94	6.58	3.09	0.114
Modern (Mod)	4.93	6.52	2.88	0.097
Contrast, p-value				
Tr vs Mod	0.267	0.856	0.920	0.781
Orig-trad+Tr-nopast vs Tr-si	0.573	0.084	0.581	0.103
Orig-trad vs Tr-nopast	0.572	0.425	0.917	0.558

[1]SCS = log2 (SCC * 1,000/100,000) + 3

Differences between LSMs of dairy system for UH traits were negligible. The milk of breeds with higher MY (BS, HF and Si) presented low lactose content (4.95, 4.96 and 4.94%, respectively). However, we found the lowest value of lactose (4.83%) in milk from Jer, a small breed with low milk production but high milk quality. Probably, the milk of this breed is characterized by a higher mineral content. For pH and lactoferrin, one contrast (Si vs GA+Ren for pH and BS vs HF for lactoferrin) reported a relevant p-

value ($P<0.01$). Not relevant differences between breeds have been observed for the SCS trait. Finally, the different dairy system management seems to have a negligible effect on UH traits.

CONCLUSIONS

In conclusion, relevant differences have been highlighted between the six breeds regarding the milk composition traits, while breed differences for UH traits were negligible. The dairy system management revealed a limited influence on all considered traits. However, the average values of milk components have shown some differences: in particular, Tr-si produces milk with higher fat, protein and casein content, but also with higher content in somatic cells.

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Milk coagulation ability of Rendena and Holstein-Friesian cattle breeds

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ABSTRACT

Aim of this study was to compare Holstein-Friesian (HF) and Rendena (RE) cattle breeds for milk coagulation and composition traits predicted by mid-infrared spectroscopy using official milk recording samples of 28 single-breed dairy herds of northeast Italy. Individual milk samples (n = 3,622) from 1,786 cows were analyzed for rennet coagulation time (RCT, min), curd firmness (a_{30}, mm), and composition traits. A linear mixed model was used to study the effect of breed and environmental factors on RCT and a_{30}. Breed was the most important source of variation for coagulation traits. In particular, milk from RE coagulated earlier and showed a firmer curd than milk from HF cows. Rennet coagulation time was shortest at the beginning of lactation, and a_{30} was better at the beginning and end of lactation. In conclusion, RE produced milk more suitable for cheese processing than that of HF. Clotting characteristics of RE breed should be considered when developing strategies useful for the valorization of this local genetic resource.
(Keywords: milk coagulation ability, dairy cattle breed, mid-infrared spectroscopy)

INTRODUCTION

Milk coagulation properties **(**MCP**)** are important in cheese-making production, especially in countries where dairy industry is based on traditional products and is market-oriented (*Cassandro,* 2003). Milk that aggregates and forms a firm curd soon after the addition of the clotting enzyme is expected to produce higher cheese yield than milk with poor coagulation properties (*Bynum and Olson,* 1982; *Riddell-Lawrence and Hicks,* 1989). Several studies have confirmed that MCP are useful information for cheese processing, yield and quality at the laboratory (*Alipanah and Kalashnikova,* 2007; *Penasa et al.,* 2010) and industrial level (*Bynum and Olson,* 1982; *Summer et al.,* 2003; *De Marchi et al.,* 2008). Moreover, MCP influence the sensory properties of cheese (*Martin et al.,* 1997).

Several approaches can be used to determinate MCP (*O'Callaghan et al.,* 2002; *Fagan et al.,* 2007; *Klandar et al.,* 2007). Among mechanical tools, the Formagraph and the Computerized Renneting Meter have been the most used to determine MCP (*Cassandro et al.,* 2008). The output are firmness/time graphs that report measures of rennet coagulation time (RCT), which is the interval, in minutes, between the addition of the rennet to milk and the beginning of coagulation, and curd firmness (a_{30}), which corresponds to the width of the graph 30 min after rennet addition. The main disadvantages of the aforementioned instruments are the limited number of samples

processed per hour, the costs related to sample processing and the skilled personnel involved. Therefore, mid-infrared spectroscopy (MIRS) offers quick and low-cost analysis, minimal sample preparation, and the opportunity to be implemented routinely to predict economically important traits such as fat, protein, casein and MCP (*De Marchi et al.,* 2009, 2012).

Differences in MCP among breeds (*e.g., Macheboeuf et al.,* 1993; *Malacarne et al.,* 2006; *De Marchi et al.,* 2007) and among cows within the same breed *(e.g., Ikonen et al.,* 2004; *Cassandro et al.,* 2008*)* have been reported. Most studies have measured coagulation properties on milk from cosmopolitan breeds such as Holstein-Friesian (HF) and Brown Swiss, whereas few studies have focused on local breeds (*Chiofalo et al.,* 2000; *De Marchi et al.,* 2007*).*

Among the latter, Rendena (RE) is a dual-purpose (milk and meat) alpine breed mainly reared in northeast Italy, with 4,066 cows undergoing milk recording (*AIA,* 2013). Rendena is a small-sized cow that exhibits good grazing ability, longevity and fertility (*Mantovani et al.*, 1997). To our knowledge, no studies have attempted to predict the coagulation ability of milk from RE breed using MIRS and repeated records per cow. Therefore, the objective of this work is to compare two cattle breeds, one local (RE) and one cosmopolitan (HF), for predicted MCP using individual milk samples collected during routine milk recording.

MATERIAL AND METHODS

Data

The data consisted of 3,622 individual milk samples collected between September and December 2011 from 20 and 8 single-breed herds of Holstein-Friesian (HF; n = 1,330 cows) and Rendena (RE; n = 456 cows) cattle breeds, respectively. Farms were located in Veneto region (northeast Italy) and were enrolled in the official monthly test-day milk recording system.

Milk samples were analyzed in the laboratory of the Breeders Association of Veneto region (Padova, Italy) using Milko-Scan FT6000 (Foss Electric A/S, Hillerod, Denmark) for fat, protein, casein and lactose contents, somatic cell count (SCC) and pH. Milk coagulation traits, namely rennet coagulation time (RCT, min) and curd firmness ($a_{30,}$ mm), were predicted by MIRS using models developed by *De Marchi et al. (*2012*)* and implemented on the Milko-Scan FT6000; those authors obtained coefficients of determination of cross-validation of 0.76 and 0.70 for RCT and a_{30}, respectively *(De Marchi et al.,* 2009, 2012*)*. Somatic cell score (SCS) was obtained via base-2 log-transformation of SCC as: $SCS = 3 + \log_2(SCC/100{,}000)$.

Statistical analysis

Data were analyzed using the MIXED procedure of SAS (*SAS Institute*, 2012) according to the following linear model:

$$y_{ijklmno} = \mu + B_i + H_j(B_i) + M_k + DIM_l + P_m + (B \times DIM)_{il} + (B \times P)_{im} + cow_n(B_i) + \varepsilon_{ijklmno},$$

where $y_{ijklmno}$ is the dependent variable (RCT or a_{30}); μ is the overall intercept of the model; B_i is the fixed effect of the *i*th breed of the cow (*i* = HF, RE); $H_j(B_i)$ is the fixed effect of the *j*th herd (*j* = 1 to 28) nested within the *i*th breed; M_k is the fixed effect of the *k*th month of sampling (*k* = September, October, November, December); DIM_l is the fixed effect of the *l*th class of stage of lactation of the cow (*l* = 1 to 12, the first being a class from 5 to 35 d, followed by 10 classes of 30 d each, a class of 45 d, and an open

class beyond 350 d, respectively); P_m is the fixed effect of the *m*th parity of the cow (*m* = first, second, third, fourth, and fifth and later parities); $(B \times DIM)_{il}$ is the fixed interaction effect between breed and DIM; $(B \times P)_{im}$ is the fixed interaction effect between breed and parity; $cow_n(B_i)$ is the random effect of the *n*th cow (*n* = 1 to 1,786) nested within the *i*th breed N ~ (0, $\sigma^2_{cow(B)}$); and $\varepsilon_{ijklmno}$ is the random residual N ~ (0, σ^2_ε). Significance of breed effect was tested on the cow within breed variance.

RESULTS AND DISCUSSION

Descriptive statistics and significance of fixed effects

Table 1 shows descriptive statistics of MCP, composition traits, and milk yield. Rennet coagulation time and a_{30} averaged 20.59 ± 3.99 min and 22.00 ± 8.81 mm, respectively.

Table 1

Descriptive statistics

Trait[a]	Mean	SD	P1[b]	P99[b]
RCT (min)	20.59	3.99	10.62	29.11
a_{30} (mm)	22.00	8.81	5.11	46.39
Fat content (%)	3.80	0.70	2.35	5.67
Protein content (%)	3.47	0.44	2.65	4.68
Casein content (%)	2.75	0.37	2.04	3.75
Lactose content (%)	4.79	0.22	4.16	5.20
SCS	4.75	1.36	2.30	8.31
pH	6.61	0.08	6.43	6.79
Milk yield (kg d^{-1})	25.68	9.96	5.80	49.60

[a]RCT, rennet coagulation time; $a_{30,}$ curd firmness 30 min after rennet addition; SCS somatic cell score

[b]P1, 1st percentile; P99, 99th percentile

Significance of fixed effects included in the analysis of RCT and a_{30} are reported in *Table 2*.

Table 2

***F*-values and significance of fixed effects included in the analysis for milk coagulation traits (RCT, rennet coagulation time; $a_{30,}$ curd firmness 30 min after rennet addition)**

Effect[a]	RCT, min	a_{30}, mm
Breed (B)	111.60^{***}	95.72^{***}
Herd(B)	6.45^{***}	5.00^{***}
Month	55.63^{***}	77.29^{***}
DIM	36.11^{***}	16.22^{***}
Parity (P)	4.63^{***}	8.99^{***}
B x DIM	1.81^{*}	1.43^{ns}
B x P	2.24^{ns}	2.78^{*}
RSD	3.39	7.84

[a]DIM, days in milk; RSD, residual standard deviation; ns = not significant; *P<0.05; ***P<0.001

Breed, herd within breed, month, DIM and parity were highly significant (P<0.001) in explaining the variability of MCP. The interaction effect between breed and DIM was significant (P<0.05) in explaining the variation of RCT but not of a_{30}. Finally, the interaction effect between breed and parity was found significant (P<0.05) for a_{30} but not for RCT.

Least square means

Milk from RE cows coagulated 2.37 min earlier and curd was 5.17 mm firmer than milk from HF cows (P<0.001). The HF breed had the worst coagulation properties as reported by *De Marchi et al. (*2007*)* who studied the variation of MCP determined by Formagraph in bulk milk. It is important to emphasize that these are the first results for MCP predicted by MIRS at individual level in RE breed.

Figure 1 shows the least squares means of MCP for HF and RE breeds across DIM. Rennet coagulation time was shortest in early lactation, and RE performed better than HF across DIM. Curd firmness exhibited the best values at the beginning and end of lactation, and it was higher in milk of RE than HF cows. The DIM effect was an important source of variation for MCP (P<0.001), and the trend of RCT and a_{30} during the first part of lactation is very similar to those reported by several authors (*Ostersen et al.,* 1997; *Tyrisevä et al.,* 2004; *De Marchi et al.,* 2007; *Penasa et al.,* 2014).

Figure 1

Least square means (with standard errors) of (a) rennet coagulation time (RCT, min) and (b) curd firmness 30 min after rennet addition (a_{30}, mm) of cows of different days in milk (DIM) and breeds (– – = Holstein-Friesian; ——— = Rendena)

a) b)

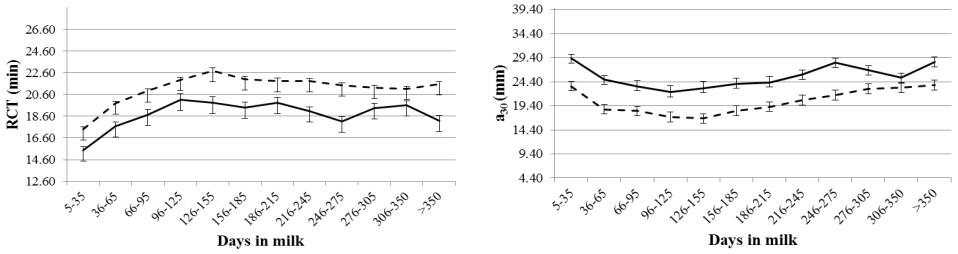
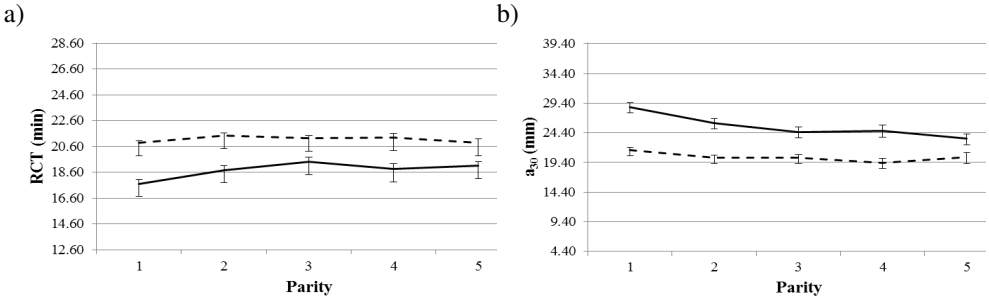


Figure 2 depicts the least squares means of MCP for HF and RE breeds across parities. Overall, RCT and a_{30} were better in primiparous than multiparous cows, and RE performed better than HF. Parity had a strong effect on MCP (P<0.001). However, the effects of parity on RCT and a_{30} are contradictory. Milk coagulation properties deteriorated with parity in the study of *Tyrisevä et al.* (2003) and *Penasa et al.* (2014), confirming findings of the present work, whereas *Ikonen et al.* (2004) reported lower values of curd firmness for primiparous than multiparous cows. Finally, *Ikonen et al.* (1999) and *Tyrisevä et al.* (2004) did not observe any effects of parity on milk clotting ability.

Figure 2

Least square means (with standard errors) of (a) rennet coagulation time (RCT, min) and (b) curd firmness 30 min after rennet addition (a30, mm) of cows of different parities and breeds (– – = Holstein-Friesian; ——— = Rendena)



CONCLUSION

Milk coagulation properties were affected by several factors, especially cow breed and environmental factors. Rendena produced milk which coagulated earlier and exhibited a firmer curd than that of HF. This result suggests that small-sized local breeds such as RE are often interesting for traits of economic importance. Besides cow breed, month, stage of lactation and parity were also important on MCP. Further research is needed to investigate the effects of farm characteristics on the variation of MCP to identify technical solutions which could help farmers to improve MCP and the opportunity for the conservation of endangered resources.

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Variation of major mineral contents in Mediterranean buffalo milk and application of Fourier Transform Infrared spectroscopy for their indirect prediction

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ABSTRACT

Minerals are important for many physiological functions and those contained in milk are actively involved in cheese manufacture. Moreover, milk minerals content is considered an indirect index of udder health in dairy cows. The aims of this study were to characterize mineral components (i.e., calcium, phosphorus, magnesium and potassium) in buffalo milk, to investigate their sources of variation and to test the effectiveness of Fourier Transform Infrared spectroscopy (FTIR) for their indirect prediction. A total of 173 buffaloes reared in five herds were milk sampled once during morning milking. Samples were analysed for calcium, phosphorus, potassium and magnesium within 3 h from collection using Inductively Coupled Plasma (ICP-OES). MilkoScan FT2 (Foss, Hillerød, Denmark) was used for the acquisition of milk spectra over the spectral range from 5000 to 900 wavenumber × cm-1179. Buffalo milk minerals (mg/L of milk) averaged 1,620, 144, 1,172 and 857 for calcium, magnesium, phosphorus and potassium, respectively. Herd and days in milk were the most important sources of variation for the former traits. Parity slightly affected only calcium and potassium. Coefficients of determination between the predicted and measured values in cross-validation (1-VR) were 0.71, 0.70, 0.72 for calcium, magnesium and phosphorus, respectively, whereas potassium exhibited a low accuracy (1-VR = 0.55). Our findings indicate that FTIR predictions could be used to assess buffalo milk components applying this rapid and non-invasive technique in the dairy industry and at the population level for breeding purposes.
(Keywords: buffalo milk, mineral content, FTIR spectroscopy)

INTRODUCTION

Buffalo milk production represents the 12% of the milk manufacture of the world, for this reason it is second after cow milk production (*Iqbal et al*., 2011). Milk is considered an important source of minerals (*Singh and Sachan*, 2011). Considering the manufacturing of milk, calcium and phosphates have an important role in rennet coagulation of milk, in the structure of the cheese and cheese yield (*Ariota et al*., 2007; *Lucey and Fox*, 1993). Also, the concentrations of many minerals are altered during mastitis (*Eshratkhah et al*., 2012; *Ahmad et al*., 2007), and these changes could be interesting to determine the manufacturing quality of the milk and for the diagnosis of subclinical mastitis. At present, one of the existing methodologies to determine mineral content in milk is Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES).

This method is too expensive for routine analysis of milk samples collected (*Soyeurt et al*., 2009). The current tool used to measure the major milk components during regular milk recording is FTIR spectroscopy, that has been proposed also to predict innovative phenotypes as fatty acid profile (*Bastin et al*., 2011), milk coagulation properties (*Bittante et al*., 2012), lactoferrin (*Soyeurt et al*., 2012) and mineral profile in bovine milk (*Soyeurt et al*., 2009). Recently a study conducted by *Ferragina et al*. (2013) on FTIR predictions for cheese yield and nutrient recovery traits showed the possibility to employ this technology to help selecting cows in dairy populations. To our knowledge there are no specific FTIR spectroscopy studies on the mineral content of buffalo, as there are no studies regarding the monitoring of milk mineral salts during lactation in buffalo. Thus, the aims of this study were to assess the variation of the mineral content in buffalo milk, to estimate sources of environmental variation during lactation and to test the possibility of using FTIR spectroscopy for the indirect evaluation of the mineral content in buffalo milk.

MATERIALS AND METHODS

Collection and analysis of milk samples

A total of 173 buffaloes were sampled once in five herds located in northern Italy from January to May 2013. Individual milk samples were collected, without preservative, during the morning milking. Samples were stored in portable refrigerators (4 °C) and transferred to the milk quality laboratory of the Department of Agronomy Food Natural resources Animals and Environment (DAFNAE) of the University of Padova (Legnaro, Italy). Samples were analysed for mineral contents within 3 hours from collection. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Ciros Vision EOP, SPECTRO Analytical Instruments GmbH, Kleve, Germany) was used as reference method to determine milk calcium, phosphorous, potassium and magnesium.

FTIR spectral collection and calibration

Buffalo milk samples spectra were collected using a MilkoScan FT2 (Foss, Hillerod, Denmark) over the spectral range from 5000 to 900 wavenumber × cm-1179. The spectra were stored as absorbance (A) using the transformation A = log(1/T), where T is the transmittance. Two spectral acquisitions were carried out for each sample, and the results were averaged prior to data analysis. As described in detail by *Ferragina et al*. (2013), calibration models were developed using the WinISI II software (Infrasoft International LLC, State College, PA) and carried out using partial least-square regression (PLS) as the chemometric algorithm. The accuracy of the model was evaluated using R^2 according to *Williams* (2003).

Statistical analysis

Sources of variation of mineral components (Ca, Mg, P and K) and milk yield (kg/d) were investigated using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) according to the following linear model:

$$Y_{ijkl} = \mu + DIM_i + Parity_j + Herd_k + e_{ijkl,}$$

where Y_{ijkl} is the observed trait (minerals: calcium, magnesium, phosphorus and potassium; milk yield); μ is the overall intercept of the model; DIM_i is the fixed effect of the *i*th class of DIM (i=1 to 6; class 1, <30 days (40 samples); class 2, 30–60 d (24 samples); class 3, 60–120 d (28 samples); class 4, 120–180 d (27 samples); class 5, 180–240 d (59 samples); class 6, >240 d (26 samples)); $parity_j$ is the fixed effect of the *j*th parity of the

buffalo (j=1 to 4 or more); $Herd_k$ is the fixed effect of the *k*th herd (k=1 to 5); e_{ijkl} is the residual random error term ~ N (0, σ^2_e).

RESULTS AND DISCUSSION

Statistics of buffalo milk mineral contents

Descriptive statistics of major mineral contents and production traits of Mediterranean buffalo milk are summarized in *Table 1*. Calcium is the predominant mineral, followed by phosphorus, potassium and magnesium. Mineral values are different from the study conducted by *Soyeurt et al*. (2009) in cow milk, where potassium was the predominant mineral, followed by calcium, phosphorus and magnesium. This dissimilarity could be attributed to the species considered and to the different approach employed to determine minerals. Nevertheless these results are in agreement with literature.

Table 1

Descriptive statistics of major mineral contents and production traits of Mediterranean buffalo milk (n=173)

Trait	Mean	P1[1]	P99[2]	CV, %
Minerals, mg/L of milk				
Calcium	1,619	909	2,378	19
Magnesium	144	82	247	25
Phosphorus	1,172	672	1,699	20
Potassium	857	453	1,203	20
Production traits				
Milk yield, kg/d	5.93	0.8	13	48
DIM, d	147.5	6	400	71

[1]P1 = 1th percentile; [2]P99 = 99th percentile

In fact buffalo milk is generally characterized by high content of calcium (180–210 mg/100 g of milk) and phosphorus (120–140 mg/100 g of milk). Calcium and phosphorus are also the main mineral milk salts mostly related with the cheese yield and rennet coagulation properties (*Ariota et al*., 2007). In particular, milk phosphorus seems to have great relevance to favour cheese yield, especially in relation to the curd hydration, while calcium is related to RCT and gel firmness (*J.A. Lucey*, 1993). Results from ANOVA (*F*-values and significance) for major mineral contents in buffalo milk and single test-day milk yield (MY) are summarized in *Table 2*.

Table 2

Results from ANOVA (*F*-values and significance) for major mineral contents and single test-day milk yield (MY) in Mediterranean buffalo milk

Minerals, mg/L of milk	Herd	DIM	Parity	R^2, %	RMSE[1]
Df	4	5	3		
Calcium	11.56***	2.86*	3.11*	47	234.25
Magnesium	5.28***	8.17***	1.48^{ns}	47	28.03
Phosphorus	19.88***	2.18*	2.25^{ns}	55	163.88
Potassium	12.91***	4.48***	2.96*	50	127.12
MY, Kg/L	26.25***	15.9***	0.48^{ns}	49	2.08

[1]RMSE = root mean square error

The herd effect was relevant in explaining the variation of all minerals (P<0.001). Days in milk affected all the considered traits. Among mineral salts, Mg and K have been highly influenced (P<0.001), while Ca and P have been less affected by this factor (P<0.05). DIM considerably influenced also milk yield (P<0.001). Calcium averaged 1,713 mg/L in the first 30 days of lactation and increased of 26.50 mg/L through the period. Phosphorus increased from about 1,194 mg/L to about 1,252 mg/L in the last class of DIM, so generally it raised of 58.01 mg/L. Potassium slightly decreased from about 944 mg/L to around 816 mg/L, so it decreased of 128.18 mg/L throughout the lactation period. Magnesium raised from about 126 mg/L in the first 30 days of lactation to just over 170 mg/L in the last days, with a trend sharply opposite to the lactation curve. In fact it increased of about 46 mg/L through lactation period. Commonly, the variability in mineral content regards especially calcium and phosphates (*Ariota*, 2007). At the beginning of the lactation period, precisely around parturition, occur some physiological changes in mineral composition. Actually the calcium concentration in colostrum is much higher than that of normal milk and near the end of lactation (*Gaucheron*, 2005). Parity did not affect minerals and milk yield, except for calcium and potassium ($P < 0.05$) that tend to decrease in buffaloes with more than 4 lactations.

FTIR predictions for mineral contents in buffalo milk

Fitting statistics of predictions models for major mineral contents in Mediterranean buffalo milk is summarized in *Table 3*. For each mineral component different math treatment (e.g. MSC)/model combinations (PLS, MPLS etc.) were explored in order to retain the best calibration equation. The coefficient of determination of cross-validation (1-VR) was used as model choice criteria.

Table 3

Fitting statistics of predictions models for calcium (Ca), magnesium (Mg), phosphorus (P), and potassium (K) contents in Mediterranean buffalo milk

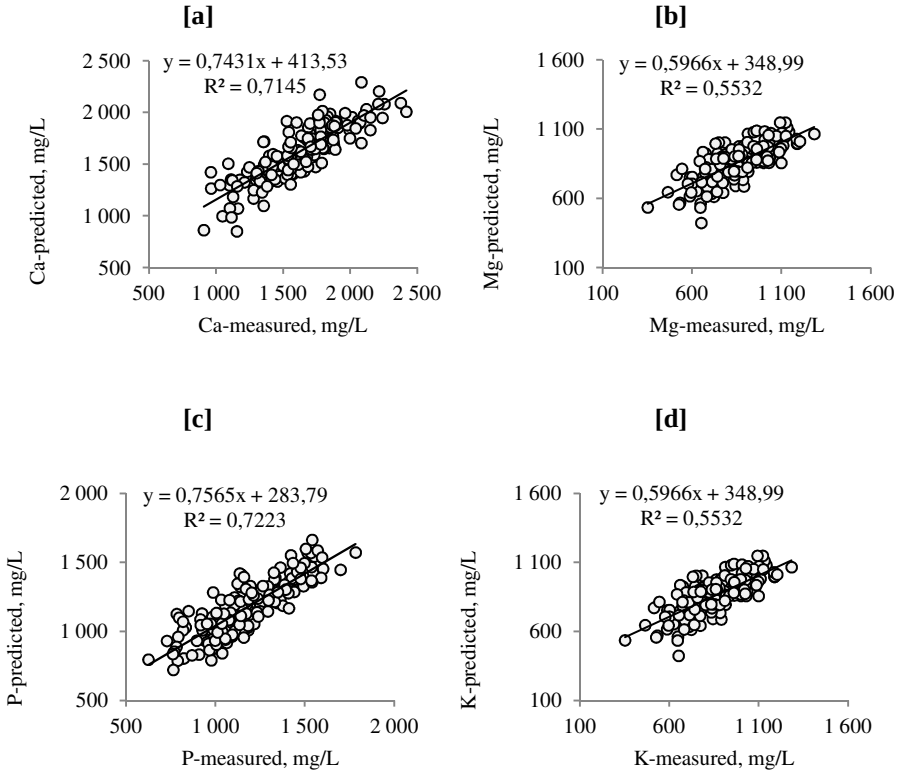
Trait	N[a]	#L[b]	Math[c]	SD[d]	SEC[e]	R^2 [f]	SEC_{cv} [g]	1-VR[h]	SEP(C)[i]
Ca	167	10	W,1,4,4,1	306	136	0.80	163	0.71	212
Mg	163	10	W,1,4,4,1	33	14	0.80	18	0.70	23
P	167	9	W,1,4,4,1	232	107	0.79	122	0.72	159
K	167	5	A,MSC, 0,0,1,1	167	102	0.63	112	0.55	145

[a]N = number of samples used in the calibration after removing outlier.
[b]#L = number of partial least square components.
[c]Math = mathematical treatments of the spectral data where the letters indicate the spectral range used for calibration (A= all the spectrum 5,011–930 cm^{-1}; W = spectra segments used 5,011–3,673 cm^{-1} 3,048–1,701 cm^{-1} and 1,582–930 cm^{-1}), MSC= multiplicative scatter correction, the first number is the order of the derivative, the second number is the segment length in data points over which the derivative was taken, the third and fourth numbers are the segment length for first and second smoothing respectively.
[d]SD = standard deviation.
[e]SEC = standard error of calibration.
[f]R^2 = coefficient of determination of calibration.
[g]SEC_{cv} = standard error of cross-validation.
[h]1-VR = coefficient of determination of cross-validation.
[i]SEP(C) = standard error of prediction corrected for the bias.

The coefficient of determination of cross-validation (1-VR) of the predicted versus measured values of mineral contents was good for calcium, magnesium, and phosphorus, and low for potassium (*Figure 1*). *Soyeurt et al*. (2009) found 0.97 R^2 for calcium and 0.88 R^2 for phosphorus in bovine milk. This could be due to the differences in terms of number of samples used for building the calibrations equations, sampling conditions and milk composition (buffalo vs cow).

Figure 1

Scatter plots of predicted vs measured values of calcium (Ca) [a], magnesium (Mg) [b], phosphorus (P) [c] and potassium (K) [d] contents (mg/L of milk) in buffalo milk



CONCLUSIONS

The present study investigated the variation of the mineral content in buffalo milk, the sources of environmental variation during buffalo lactation and the using of FTIR spectroscopy for the indirect evaluation of minerals in buffalo milk. According to the literature, calcium was the predominant mineral, followed by phosphorous, potassium and magnesium. Herd and DIM were the most important sources of variation for the considered traits, as expected. Parity slightly affected only calcium and potassium. R^2 for cross-validation of the predicted versus measured values of mineral contents was good

for calcium, magnesium and phosphorus. Potassium revealed the worst fitting value. Ca, Mg and P predictions could therefore be useful for the efficient monitoring of health status of dairy populations, besides being a rapid and cheap tool for improving the nutritional quality of milk. Future work is necessary to examine the economic importance of these traits and their improvement in dairy populations.

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Effect of cheesemaking with microparticulated whey proteins on the concentration of low molecular thiols in cheese

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ABSTRACT

Aim of this work was to investigate different concentrations of microparticulated whey proteins (MWP) added during cheesemaking process on the recovery of low molecular weight thiols (LMT) in cheese. Historically, milk whey has been considered an industrial waste, because of its high cost of disposal and its polluting potential. In recent years the re - utilization of this waste represents an interesting perspective. Nowadays, several procedures are available to whey constituents recovery: some of the main are whey proteins (WP) and LMT. Whey ultrafiltration is the most common treatment to WP recovery. Thereafter, the WP undergo processes that lead to the production of protein aggregates (microparticulated-MWP), useful in different sectors of the food industry for their high gelling power and potential in LMT linkage. Mini-cheesemaking trial using milk standardized at 3.5% of protein with 3.0% or 4.0% MWP were carried out. Cheesemaking were performed in 6 days, 3 days for each treatment. Within a day, 3 replicates of the same treatment were carried out (n=18). The LMT of milk and whey were determined using RP - HPLC, while LMT in cheese were calculated by difference. Data were analyzed through a generalized linear model as fixed effects of the MWP concentration, replicate, and day of cheesemaking nested within MWP. Results showed that the quantified concentration of LMT in cheese were quite stable in both the percentages of MWP. The soluble properties of LMT represent a problem in their recovery in cheese; indeed beyond a certain concentration they are not retained in the curd, but are released in the whey.

(Keywords: microparticulated whey proteins, RP - HPLC, thiols, whey protein)

INTRODUCTION

Animal cells produce energy by reducing molecular oxygen in water. This process generates few amounts of free radicals called reactive oxygen species (ROS), that can damage the chemical structure and the biological function of cells molecules (*Droge,* 2002; *Siliprandi et al.,* 2008). The negative effects of ROS are on a large scale: they mainly act on lipid peroxidation of plasma membrane, oxidative alterations of proteins, up to the DNA cleavage. Cells have developed several mechanism to remove and inactivate ROS, by producing antioxidants. The molecules that act as antioxidants are enzymatic (catalase, superoxide dismutase and glutathione peroxidase) or non - enzymatic. Among the aforementioned, the main are fat - soluble vitamins A and E,

ascorbic acid and glutathione (GSH) in the cytosol (*Robbins et al.,* 2008). In particular, GSH, tripeptide composed of glycine, glutamate and cysteine, belonging to the class of low molecular thiols (LMT), plays a central role in deactivation of ROS (*Fang et al*., 2002).

Milk whey, a by - product of cheesemaking, has a great antioxidant activity, mainly due to its content in whey proteins (WP) rich in cysteine, key element in the GSH biosynthesis. The whey protein concentrates (WPC) and aggregates (MWP) produced by ultrafiltration and microparticulation of whey, have attracted the scientific community attention because of their higher content in WP (*Hakkak et al.,* 2000). Nowadays, several studies have been carried out to increase GSH production by WP and WPC administration in immunodeficient patients with HIV infection. Products based on WP have been used as a cystein source to increase intracellular levels of GSH production (*Micke et al.,* 2002).

This study aimed to investigate the variation of LMT, cysteine (Cys), cystein – glycine (Cys - Gly), γ–glutamylcysteine (γ – GC), and GSH, after MWP addition during cheesemaking process.

MATERIALS AND METHODS

Bulk milk used for cheese making was collected in the Soligo dairy cooperative (Soligo, Italy) added using 3.0% or 4.0% of MWP and standardized at 3.5% of proteins using proper quantity of milk protein concentrate (MPC). Samples of MWP and MPC were obtained in the same dairy, after ultrafiltration by polyethersulphone membrane (10,000 Da; TetraPak Food Engineering, Lund, Sweden) at 10°C, and than treated at 95°C for 10 min, at 40 bar of pressure. The milk used in each day was analysed with MilkScan FT2 (Foss Electric, Hillerod, Denmark).

Ten litres of standardized milk has been used for mini - cheesemaking. Coagulation was monitored with sensor CoAguLite (Reflectronics Inc., Lexington, KY; *Fagan et al.,* 2007). Milk is added to a freeze–dried starter culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (TB, MicroMilk, Crema, Italy) and to a solution of commercial liquid rennet (chymosin 75:25 bovine pepsine, rennet De Longhi Michele & CSas, Treviso, Italy) diluted in water (1:3). Finally the crud is placed in a mold and is incubated at 37°C for about 3 hours, until pH is under 5.5. The form is placed in a saline solution for one hour (1.14 kg of NaCl per L) and then in chilled room for curing (10 days at 4°C and 85% of relative humidity). Cheesemaking were performed in 6 days, 3 days for each treatment. Within a day, 3 replicates of the same treatment were carried out (n=18).

Soluble LMT quantification in milk and whey was carried out by RP–HPLC method, using C18 column, after the application of a derivatization protocol as proposed by *Masi et al.* (2002). LMT concentration in cheese was calculated by difference between milk and whey LMT. The normal distributions of LMT in milk, whey and cheese were checked using Shapiro-Wilk's Test. Data regarding concentrations of LMT in milk, whey and cheese were analysed with a linear model, using the GLM procedure of SAS (SAS 9.2, 2008). The model included the fixed effect of the MWP concentration (2 levels), replicate (3 levels) and day of cheesemaking nested within MWP. The effect of MWP concentration has been tested on error line within day variance. Bonferroni's test was used in order to determine differences between LMT means concentration. Significance was established at $P \leq 0.05$.

RESULTS AND DISCUSSION

The effect of cheesemaking with MWP was tested to assess any changes of the LMT concentrations in milk, whey and cheese. In *Table 1* are summarized the descriptive statistics of thiols in milk added with 3 and 4% of MWP, whey and Caciotta cheese obtained after cheesemakings. The results of the Shapiro-Wilk's test showed for all traits a normal distribution of data. The cysteine was the most abundant thiol in milk as well in whey and cheese. The high concentration of cysteine in milk and whey has been reported by several authors (*Parodi,* 1998; *Bounous,* 2000). While, in the same matrices γ – GC and GSH showed lower concentrations. Finally, the Cys – Gly, was more concentrated in milk, but at the end of the cheesemaking process it is found in the whey. For this reason it has fairly low levels in cheese.

Table 2 shows the results of the variance analysis conducted for the LMT present in milk, whey and cheese. The effect of the date is highly significant in explaining the variability of the γ – GC in whey, and Cys – Gly in milk and cheese. Such daily variability could be due to different LMT concentration in the starting milk. For all the studied traits, no statistically significant effects are observed for replicate and MWP.

In *Table 3* are reported the least squares means of milk, whey and Caciotta cheese thiols across different concentrations of MWP (3.0% and 4.0%). LMT concentration in milk was not affected by the two MWP treatments, except for the γ – GC. While, in whey the concentration of thiols, γ – GC and GSH, was affected by MWP. By increasing concentration of MWP an increment in both thiols losses was observed. In cheese, MWP treatment did not affect thiol concentration. All LMT in all matrices analyzed, evidenced an increasing trend by increasing percentage of MWP used. The soluble properties of LMT (*Guttemberger et al.,* 1992) had reduced their potential recovery on cheese. Adjustments in MWP production, to retain major LMT, and during cheesemaking, should be made to improve the LMT concentration in cheese produced with MWP.

Table 1

Descriptive statistics of milk, whey and Caciotta cheese thiols for both treatments (n=18)

Thiols[1], µM	Mean	SD	Minimum	Maximum
Milk				
Cys	33.24	3.86	27.93	43.41
Cys – Gly	1.35	0.23	1.08	1.93
γ – GC	0.54	0.22	0.23	0.84
GSH	0.81	0.28	0.43	1.58
Whey				
Cys	26.73	3.53	19.22	32.15
Cys – Gly	1.15	0.16	0.90	1.60
γ – GC	0.43	0.24	0.14	0.80
GSH	0.54	0.22	0.16	1.05
Cheese				
Cys	6.51	4.17	0.33	15.43
Cys – Gly	0.19	0.15	0.00	0.52
γ – GC	0.11	0.08	0.00	0.25
GSH	0.26	0.20	0.03	0.65

[1]Cys=Cysteine; Cys–Gly=Cysteine–Glycine; γ–GC=γ–Glutamylcysteine; GSH=Glutathione

Table 2

Results from ANOVA (*F* - value and significance) for milk, whey and Caciotta cheese thiols

Thiols[1], µM	Effect			RMSE[4]	R^2
	Date (MWP)[2]	Replicate	MWP[3]		
Milk					
Cys	0.86	1.15	1.28	3.88	0.41
Cys – Gly	6.47**	0.47	0.01	0.15	0.73
γ – GC	16.49**	1.33	0.48	0.10	0.88
GSH	2.08	1.12	1.32	0.26	0.51
Whey					
Cys	0.93	0.27	0.12	3.84	0.30
Cys – Gly	1.20	1.71	0.02	0.15	0.45
γ – GC	31.09***	1.81	0.26	0.08	0.93
GSH	2.87	0.88	2.29	0.16	0.66
Cheese					
Cys	0.23	0.94	1.53	4.74	0.24
Cys – Gly	9.45**	1.62	0.01	0.09	0.80
γ – GC	1.41	0.20	0.18	0.08	0.39
GSH	1.35	0.74	0.06	0.20	0.41

[1]Cys=Cysteine; Cys–Gly=Cysteine–Glycine; γ–GC=γ –Glutamylcysteine; GSH=Glutathione
[2]Data(MWP) = Data of analysis nested on MWP
[3]MWP = microparticulated whey proteins 3.0% and 4.0%
[4]RMSE = root mean square error
***P* < 0.01; ****P* <0.001

Table 3

Least squares means of milk, whey and Caciotta cheese thiols across different concentrations of MWP[1]

Thiols[2], µM	MWP 3%	MWP 4%
Milk		
Cys	32.28[a]	34.20[a]
Cys – Gly	1.35[a]	1.35[a]
γ – GC	0.48[a]	0.61[b]
GSH	0.72[a]	0.90[a]
Whey		
Cys	26.43[a]	27.03[a]
Cys – Gly	1.15[a]	1.16[a]
γ – GC	0.38[a]	0.49[b]
GSH	0.44[a]	0.64[b]
Cheese		
Cys	5.85[a]	7.17[a]
Cys – Gly	0.20[a]	0.19[a]
γ – GC	0.10[a]	0.12[a]
GSH	0.28[a]	0.25[a]

[1]MWP = concentration of microparticulated whey proteins
[2]Cys=Cysteine; Cys–Gly=Cysteine–Glycine; γ – GC=γ –Glutamylcysteine; GSH = Glutathione

CONCLUSIONS

Cheesemaking trials with different MWP concentrations has not led to significant changes in the concentration of LMT as cysteine, γ - glutamylcysteine, cysteine - glycine and glutathione in cheese. Moreover the cheesemaking leads to the loss of such molecules, most of which are found in the whey. Other studies should be performed to confirm that LMT are lost in the whey after cheesemaking; in particular need to be developed further methods for the quantification of non-soluble thiols, that may be bound to whey proteins, due to glutathionylation reactions that occur in the microparticulation process.

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Stress indicators and meat quality of pigs affected by different durations of lairage time

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ABSTRACT

Objective of this study was to investigate the effect of lairage time pigs on stress indicators and meat quality. The study was performed on 90 barrows and gilts divided into three groups according to lairage time: 0 h, 6 h and 24 hours. Blood samples were taken after exsanguination and glucose, lactate, cortisol, as well as activity of creatine kinase were determined. At the slaughter line and in laboratory pH values in ham and LD muscle 45 minutes and 24 h after slaughter, as well as meat colour coordinates (L, a*, b*), drip loss, cooking loss and instrumental tenderness were measured.. Concentrations of blood stress parameters were highest in the first group (0h lairage time). Statistical analysis showed a significant effect of resting before slaughter on final pH values measured 24 hours after slaughter in ham. Moreover, resting time had a significant effect on L* and a* values and showed tendency to decrease EZ drip (%) as lairage time increased. Pigs slaughtered without rest prior to slaughter showed higher levels of stress and tend to have less desirable meat quality traits.*
(Key words: pigs, lairage time, stress, blood parameters, meat quality traits)

INTRODUCTION

Pigs are very sensitive to various types of stress, especially at the end of production before slaughter. Length of transport, i.e. the distance from the farm to slaughterhouse and non-adequate loading and unloading induces stress on both behavioural and physiological levels.

Apart the negative effect of high stress on animal welfare, meat quality could also be significantly affected. Studies showed (*Perre et al.,* 2010; *Gispert et al.,* 2000) that transport could affect meat pH values or increase the proportion of DFD (dark, firm, dry) or PSE (pale, soft, exudative) conditions. Drip loss values could also be affected (*Murray and Jones,* 1994). These undesirable effects can be diminished by allowing pigs to recover with optimum lairage time which is, according to *Nanni et al.* (2002) considered as the most important factor prior to slaughter that affects meat quality. *Warris* (2003) reported that optimal lairage time should be 1-3 hours since shorter times were usually associated with the PSE meat. On the other side, longer lairage was associated with more aggressive behaviour, more skin damage and a lower carcass yield. In addition, recommendations for length of lairage time are different regarding the country's geographical location (*Warris*, 2003). In southern European countries where temperatures in certain part of the year are very high, shorter lairage is recommended.

By measuring some of the indicators of stress like glucose, lactate, creatine kinase and cortisol from the blood, level of stress arisen before slaughter with its possible effect on important meat quality traits can be determined. *Salajpal et al.* (2005) determined that a long period of rest reduces glucose levels in blood and produces the signs of muscle tissue deterioration. *Šmeiecinska et al.* (2011) found different levels of stress in pigs slaughtered immediately after transport than those with 24 h lairage time. Although there were no differences in the incidence of PSE meat, pigs with 24h lairage time had more favourable sensory attributes of meat.

Objective of this study was to investigate the effect of lairage time on stress indicators and meat quality traits of pigs.

MATERIAL AND METHODS

Animals and experimental design

The study was performed on 90 pigs divided into three equally sized groups. The pigs were commercial hybrids, fattened to 110–120 kg live weight on family farms in the eastern part of Croatia. All farms were located approximately 100 kilometres away from the slaughterhouse. Transport of animals to the slaughterhouse was carried out in trucks with 180, 150, and 160 pigs per each load. Loading of the pigs in each of the trucks lasted for approximately 100 minutes followed with approximately 100 minutes of transport. Unloading of the pigs at the slaughterhouse lasted approximately 30 minutes. Pigs from all three groups were unloaded to slaughterhouse in the morning hours. Animals from the first group were slaughtered immediately after loading, the second group had 6 h lairage time and the third rested for 24 h prior to slaughter.

Blood samples and stress indicators

After stunning the animals with CO_2 and before incision of blood vessels and exsanguination blood samples were collected by venepuncture into two tubes. Tube without anticoagulants served for glucose, creatin-kinase activity and cortisol concentrations, while sodium fluoride-potassium oxalate tubes served for lactate determination. The concentrations of glucose (mmol/L; GLU), lactic acid (mmol/L; LA) and creatin-kinase activity (U/L; CK) were determined by automatic biochemical analyser Beckman Coulter AU400 randomly with up to 400 photometric tests per hour. The concentration of cortisol (nmol/L; COR) in pigs was measured by electrochemiluminescence immunoassay "ECLIA" (Roche Elecsys 2010, Roche Diagnostics, Mannheim, Germany). Reproducibility was determined using an Elecsys reagents six times a day, 10 days in total (n=60). The intra- and inter- assay variation coefficient were <1.1% and <1.6% for the lowest detection limit.

Meat quality traits

Meat quality traits were measured in loin (*m. longissimus dorsi* - *LD*) and ham (*m. semimembranosus* – *MS*). At the slaughter line, pH values were measured 45 minutes (pH_{45}) and 24 hours (pH_{24}) *post mortem* using digital pH-meter "Mettler MP 120-B". Drip loss was measured by "bag method" according to *Honikel* (1987) and as EZ drip (*Christensen*, 2003). Meat colour coordinates were measured 24 h *post mortem* by "Minolta CR-300" device on *LD* muscle and expressed as L*, a* and b* values. Instrumental tenderness was analysed on a 2.54 cm thick chops of *LD* muscle that were frozen, defrosted for 24 h at 4 °C, sealed in vacuum bags, cooked in water bath to 73 °C internal temperature and cooled at 4°C overnight. Shear force was measured on at least

six 1.27 mm thick chops using a TA.XTplus Texture Analyser. Cooking loss was assessed from *LD* samples used for instrumental tenderness measurement. It was calculated from weights taken before and after cooking and expressed as a percentage.

Statistical analyses

GLM procedure of Statistica for Windows software (StatSoft 2007-2010) was used to examine the effect of lairage time on stress indicators and meat quality traits of investigated pigs. The groups were compared for all traits using Tukey's range test ($p<0.05$).

RESULTS AND DISCUSSION

Effect of lairage time on stress indicators

Table 1

Least square means and standard errors (in brackets) for biochemical stress indicators in relation to lairage times

Blood parameter	Lairage time			Significance
	0h	6h	24h	
Lactate (mmol/L)	5.10^{a} (0.39)	3.95^{b} (0.30)	3.91^{b} (0.30)	*
Glucose (mmol/L)	4.68^{a} (0.10)	4.07^{b} (0.18)	4.50^{ab} (0.08)	**
Creatin-kinase (U/L)	12049.36^{a} (1767.05)	5379.76^{b} (889.69)	3085.33^{c} (448.11)	***
Cortisol (nmol/L)	248.26^{a} (21.00)	124.71^{b} (13.29)	105.32^{b} (8.93)	***

*** = $p<0.001$; ** = $p<0.01$; *=$p<0.05$; † = $p<0.1$; n.s. = not significant

As expected, results show a significant increase in the concentration of LA and COR with an increase of the lairage time. This corresponds to studies of *Hambrecht et al.* (2004) and *Merlot et al.,* 2011). Variation in glucose concentrations tend to vary depending on the level of stress prior slaughter. In our study, concentrations in GLU were the highest in a group of pigs with no rest prior to slaughter. Opposite to these results, *Perez et al.* (2002), who compared the effect of transport time (15 min and 3 h), did not find differences in GLU concentrations in investigated groups. The study of *Merlot et al.* (2011) also showed that level of stress does not necessarily effect blood glucose concentrations. In *Table 1* it can be observed that lairage time significantly affected blood CK concentration, where the highest concentration of this blood parameter was measured in group of pigs without rest and the lowest in the group with 24h rest before slaughter. These results correspond with results from other studies (*Brown, et al.,* 1997) where highest values of creatine-kinase were measured in group of pigs with minimum level of stress before slaughter, or with shorter transport (*Perez et al.*, 2002). In later case, stress was primarily induced by the loading of pigs in the slaughterhouse, which clearly affected CK concentrations. Furthermore, *Yu et al.* (2009) found an increase ($P<0.01$) of CK activity after 1h or 2h of transportation indicating muscle damage and result from disruption in muscle cell membrane (sarcolemma) function and permeability.

Effect of lairage time on meat quality traits

Table 2

Least square means and standard errors (in brackets) for meat quality traits in relation to lairage time

Trait	Lairage time			Significance
	0h	6h	24h	
pH_{45} ham	6.44 (0.02)	6.46 (0.03)	6.42 (0.03)	n.s.
pH_{45} loin	6.33 (0.02)	6.38 (0.03)	6.31 (0.03)	n.s.
pH_{24} ham	5.50^{b} (0.01)	5.48^{b} (0.01)	5.59^{a} (0.03)	***
pH_{24} loin	5.49^{a} (0.01)	5.44^{b} (0.01)	5.53^{a} (0.02)	***
Drip loss (%)	5.54 (0.46)	5.04 (0.32)	4.61 (0.50)	n.s.
EZ drip (%)	5.77 (0.53)	4.89 (0.24)	4.35 (0.48)	†
L^{*}	54.91^{a} (0.41)	53.82^{ab} (0.60)	52.75^{b} (0.47)	***
a^{*}	5.60^{b} (0.16)	5.46^{b} (0.17)	6.36^{a} (0.19)	***
b^{*}	2.26 (0.13)	2.34 (0.19)	2.66 (0.13)	n.s.
Cooking loss (%)	33.45 (0.28)	33.25 (0.26)	33.29 (0.34)	n.s.
WBSF (N)	53.16^{ab} (1.13)	49.50^{b} (1.51)	53.89^{a} (1.47)	†

*** = $p<0.001$; ** = $p<0.01$; * = $p<0.05$; † = $p<0.1$; n.s. = no significance

From *Table 2* it can be observed that lairage time did not affect pH values measured 45 minutes *post mortem* in loin nor ham. Contrary to these results *Brown et al.* (1997) found significantly higher pH_{45} values in group of pigs with usual manipulation in slaughterhouses than the group of pigs specially manipulated before slaughter. In a similar study of *Hambrecht et al.* (2004), pH values measured 30 minutes after slaughter were lower in the group of pigs with higher level of stress before slaughter.

pH values measured in ham 24 hours after slaughter were strongly influenced by rest before slaughter. As it can be seen from Table 2, pigs which rested for 24 h before slaughter exhibited favourable final pH values. Contrary to results of this study *Brown et al.* (1997) and *Perez et al.* (2009) did not find significant differences between groups of pigs treated with various kinds of stress, e.g. manipulation of animals prior to slaughter or length of transport. Results from this study could therefore suggest longer lairage time as efficient way to prevent unfavourable lowering of pH_{24}.

No significant differences between investigated pig groups were found for drip loss and EZ drip. However, a tendency of decreasing the drip loss is observable as lairage period increases. Similar results were found by *Brown et al.* (1997) and *Perez et al.* (2002). From Table 2 it can be observed that meat from pigs with 24 h lairage time exhibited the lowest L* and the highest a* values suggesting a positive effect of long

lairage time on meat colour. Contrary to our results other studies did not find differences in paleness between studied groups of pigs in regard to other stress sources (*Brown et al.,* 1997; *Hambrecht et al.,* 2005). Instrumental tenderness (WBSF) was highest in the group with 24h lairage time. Probably this is the result of calpain proteolytic enzyme system status, which was not measured in this study. Furthermore, in the case of this trait, interpretation of results could be difficult since the relation of physical stress and tenderness is still unclear.

CONCLUSION

Pre-slaughter conditions, such as lairage time affects physic-chemical blood parameters, as well as overall meat quality in pigs. Animals slaughtered immediately after unloading exhibited the highest concentrations of glucose, lactate, cortisol and activity of creatin-kinase. With extend of lairage time blood parameters tend to decrease and meat quality traits improve.

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Possibilities of branding the pork in Croatia – review

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ABSTRACT

Black Slavonian pig is an indigenous pig breed black in colour, resistant and convenient for keeping in extensive conditions. It is also characterized by good meat quality, suitable for typical traditional meat products. Traditionally produced food came into the focus which led to more often use of geographical indications as the valuable rural development strategy. Since Black Slavonian pig is a late maturing breed with low lean meat content, meat processors often use the meat of modern pig breeds in the production of traditional products. Molecular identification of breed is a good tool for authentication of meat. Since age and sex are among the most significant sources of variation of the carcass and meat quality traits in pigs, the research on the optimal time for slaughter with respect to these factors could increase the profitability of pork production. The genetic influence on meat quality traits is nowadays well described by the use of molecular markers and candidate genes such as IGF2, MC4R, H-FABP3 and LEPR; their frequencies could be of assistance in the description of Black slavonian pig and its exceptional meat quality traits. The investigation of sensory characteristics and chemical analysis of meat and meat products is needed to determine the typical physicochemical characteristics and sensory profile of pork originated from Black Slavonian Pig which is a firm base for branding. Finally, DNA characterisation of the breed can be used as the tool for authentication of pork in the aim of preventing possible adulterations of autochthonous meat products.
(Keywords: Black Slavonian pig, traditional meat products, intramuscular fat content, molecular markers)

INTRODUCTION

Black Slavonian pig is a pig breed established at the end of the 19th century near Osijek in eastern Croatia. This pig had excellent characteristics for that time, as confirmed by a gold medal won at Vienna World Exposition in 1873. The breed is black in color, resistant and convenient for keeping in extensive (pastures, woods) and half-extensive conditions (pens with some free space). A population size began to decline due to the appearance of modern breeds, superior in lean meat content and better reproductive ability. Although Black Slavonian pig has low productivity, it is also characterized by good meat quality, suitable for producing typical traditional meat products such as "kulen" (dry cured sausage spiced with red paprika), ham, bacon and other meat products. In recent years, the traditionally produced food came into the focus which led to more often use of geographical indications as the valuable rural development strategy. Geographical indication are defined in current EU legislation as protected designation of

origin (PDO), protected geographical indication (PGI) and Traditional Specialties Guaranteed (TSG). These can be a powerful tool in branding of the meat product but also fresh meat.

The objective of this paper is to discuss various scientific approaches which could help in the creation of new brand among the animal products in Croatia.

MOLECULAR IDENTIFICATION

The first criteria and the simplest one to distinguish Black Slavonian pigs from other breeds is coat color. However, phenotypic distinguishing between purebred and F1 crossbred pigs is not possible because of dominant black color of Black Slavonian pig (*Margeta et al.,* 2009). *Extension (E)* is one of the coat color loci, which encodes the melanocortin receptor 1 (MC1R) expressed in melanocytes. *Extension/MC1R* is also one of the major coat color loci in pigs and a series of alleles with phenotypic effects has been revealed by sequence analysis (*Kijas et al.,* 2001; *Giuffra et al.,* 2000). Wild boars possess wild-type alleles; *MC1R*1* (European) or *MC1R*5* (Japanese wild boar). Two different alleles for dominant black color were detected. Large Black and Meishan pigs carry *MC1R*2*, while Hampshire possesses *MC1R*3*. The recessive red coat color of swine is characteristic for Duroc and is associated with *MC1R*4*. The sixth allele, *MC1R*6* was determined in Yorkshire, Landrace and black-spotted pig breeds (*Kijas et al.,* 2001). This approach may be not sufficient to differentiate Black Slavonian pig from other black coated breeds. However, the development of DNA based markers in the last two decades has revolutionized the possibilities to monitor genetic diversity of populations by making it feasible to screen large numbers in a relatively short time. One type of marker that has been intensely used for population studies in the last 10 years, are the so-called microsatellite or single sequence repeat markers (*Martínez et al.,* 2000; *Laval et al.,* 2000). In the pig, numerous studies of genetic variation between or within different pig breeds were conducted by genotyping multiple microsatellite loci (*Li et al*., 2004, *Fang et al.,* 2005; *Swart et al.,* 2010). Recent studys have included SNP analisys in the traditional pig breed-labelled products Wilkinson et al. (2012), but also for fatty acid composition (*Revilla et al.,* 2014), intramuscular fatty acid composition (*Ramayo-Caldas et al.,* 2014), growth in purebred population (*Stratz et al.,* 2014; *Yung et. al,* 2014) etc.

INVESTIGATIONS OF GROWTH

Growth is one of the main physiological activities in all of domestic animals, but it has a special significance in the case of animals oriented at meat production such as pigs, beef, sheep, poultry etc. Traditionally, the changes of body composition during growth are studied using the methods such as dissection of the carcass, CT, MRI and some other useful techniques at varying age or body weight (*Giles et al.,* 2009). It is a complex problem, especially when it comes to a modelling of the growth patterns. If some of the dynamic, non-linear functions are selected for modelling, it is important to correctly determine the upper limit of growth, i.e. biological maximum (A) growth of an animal, mature weight or a maximum weight in the point of interest. This parameter is the measure of animal's growth capacity or potential, which is one of the determinants for the prediction of the weight of an animal in given conditions (*Wellock et al.,* 2003). *Kuhn et al.* (1985) used the Gompertz function, while López et al. (2000) used Gompertz, Richard's and the generalised Michaelis-Menten function to describe the

growth of several species. On the other hand, *Kušec et al*. (2008) showed that asymmetric S-function can be used as a tool in the prediction of live weight, muscle and fat growth of pigs not only within the interval of measurement but throughout the whole time scale. Since it is very well known that carcass composition and meat quality traits are significantly influenced by age, growth studies can improve the profitability in pig production; for example by prediction of optimal slaughter weight/age which is especially important in case of heavy pigs aimed at the production of traditional meat products.

INVESTIGATION OF CANDIDATE GENES

The genetic influence on meat quality traits is nowadays well described by the use of molecular markers by which many of the candidate genes were discovered. There are several interesting candidate genes such as MC4R, IGF2, H-FABP3 and LEPR.

MC4R

This gene plays a major role in the regulation of food intake and regulation of energy balance. Targeted alteration (mutation) of MC4 receptor evidently causes increased food intake. Porcine MC4R locus is mapped on the 1st chromosome (SSC1 q22-q27) and Asp298Asn missense mutation is identified (*Kim et al.,* 2000). This mutation is associated with pigs that grow faster and have greater fat thickness, and its effect by the number of days required to reach slaughtering weight of 110 kg, daily gain and daily food intake in different commercial pig breeds (*Hernandez-Sanchez et al.,* 2004). *Piorkowska et al*. (2009) were exploring the effect of MC4R gene on carcass composition, growth and meat quality traits of 1191 gilts originating from five different breeds. The authors found that G allele is common in breeds selected for leanness (Pietren 92.4%), and rare in breeds with the higher percentage of intramuscular fat (Duroc 31.5%). *Carrodeguas et al.* (2005) and *Burgos et al.* (2006) designed the RT-PCR analysis to explore the possibility of using MC4R and IGF2 as a selection marker that would allow the prediction of fattening capacity of Iberian pigs and Duroc, breeds used for producing hams protected by designation of origin (*Ovilo et al.,* 2006; *Schwab et al.,* 2009).

IGF-2

IGF2 (somatomedin A , insulin-like growth factor 2) is a member of insulin family of the /IGF/ relaxin growth factors which possess activity to improve tissue growth. Single nucleotide polymorphism (G to A transition) was observed in exon 2 of this gene and microsatellite (SW9) located 800bp lower than IGF2 stop codon is mapped using linkage analysis (*Nezer et al.,* 1999). Quantitative Trait Locus (QTL), which is inherited and expressed only from the paternal allele, thereby impacting on muscle mass, accumulation of fat and the heart girt, is mapped close to IGF2 gene (*Knoll et al.,* 1999). Substitution of IGF2-intron3-G3072A, localized in a highly conserved regulatory region, is causing mutation of this QTL.

H-FABP3

Studies were conducted which detected candidate genes that have an impact on the content of intramuscular fat content in pig carcasses: intracellular cardiac fatty acid-binding protein, H-FABP (*Li et al.,* 2010.), ccyl coenzyme A diacylglycerol acyltransferase (DGAT-1), sterol binding protein 1c (SREBP-1c) (*Chen et al.,* 2008),

leptin receptor. Determined percentage of intramuscular fat content in some commercial pig breeds is less than 1.5%, but the assumption is that the proportion of IMF in Black Slavonian breed ranges between 6% and more. FABP3 gene can be used as a genetic marker in the selection for the intramuscular fat content. *Tyra et al*. (2013) reported that 30 % to 35% of the IMF variation in the study populations consequence of FABP3 gene polymorphism. Research conducted by *Gerbens et al.* (2001) and *Zhao et al*. (2012) confirmed the association of polymorphisms of genes FABP3 content and intramuscular fat.

LEPR

LEPR gene is mapped on chromosome 6 and it has a known effect on fattening traits and control of food consumption in pigs. Leptin is a protein whose activity is mediated through the leptin receptor gene and LEPR gene that is considered as the candidate gene for growth traits. *Muñoz et al*. (2011) state the connection of the not synonymic mutations p.Leu663Phe with consumption of the foods and consequently, with the formation of adipose tissue.

INVESTIGATION OF SENSORY TRAITS AND CHEMICAL ANALYSIS OF MEAT AND MEAT PRODUCTS

The indigenous Slavonian dry-cured meat products obtained from meat of Black Slavonian Pig are highly recognized and most appreciated by consumers in Croatia due to specific sensory profile that originates from the meat of these pigs. Genetic background of the pigs and their traditional outdoor feeding on natural resources such as grass and forest of Slavonian oak (*Quercus robur L.*) with addition of small amount of corn or other grains (*Karolyi et al.,* 2010) are probably responsible for generation of the specific extraordinary sensory features of the autochthonic Slavonian kulen and other Slavonian traditional dry-cured meat products. Due to activity of the specific endogenous enzyme system (proteolytic and lipolytic; *Cava et al., 2004*), and the oxidative phenomena (*Gandemer,* 2002) during processing of dry-cured meat products, important degradative changes in proteins and lipids take place. Some authors have found differences in proteolytic and lipolytic activities as a consequence of genetic background (*Cava et al*., 2004) and some also have found these differences as a consequence of free-range rearing of pigs (*Daza et al.,* 2005; *Muriel et al.,* 2002). These differences lead to the formation of specific volatile and non-volatile compounds; many of them have a decisive influence on the creation of the specific aroma and flavour of the meat products.

CONCLUSIONS

Black Slavonian pig is pig breed with desirable meat quality and gives an excellent material for added value high quality products-true Croatian brands. First step in the process of branding pork is genetic description of breed, in our case Black Slavonian pig. Further investigations on growth can improve the profitability of pork production by setting the correct time for slaughter. Full description of sensory traits with the possibility of differentiation of pork originating from Black Slavonian from other, commercial breeds are also crucial in the process of branding. Finally, DNA characterisation of the breed should be used as the tool for authentication of pork in the aim of preventing possible adulterations of autochthonous meat products.

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Effect of excess folic acid on egg production, fertility and hatchability in layer breeders

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ABSTRACT

*Folic acid (**FA**) (also known as folate or vitamin B9) is essential for all tissues with a high rate of cellular division and growth and therefore very important in reproduction. The present study was planned to see the effect of FA supplementation on productive and reproductive performance of layer breeders. A total of 105 cocks of the sire line and 906 hens of the dam-line were randomly assigned to 4 floor pens in a deep litter trial and mated at a ratio of 1 cock : 8.6 hens. Two dietary treatments were used: an unsupplemented practical corn-soybean meal basal diet and the basal diet supplemented with 50 mg of FA/kg of diet. Egg production, feed intake, mortality, fertility, hatchability and related parameters were measured during 36 to 39 wk of age. Compared with birds fed control diet, excess FA supplementation reduced (P<0.0001) feed intake and percentage hen day egg production (P<0.05). Egg weight, fertility and hatchability were similar in the laying hens fed the two dietary treatments. The BW of the newly hatched chicks was increased (P<0.05) with the supplementation of FA to the diet when compared with the control treatment. Percent mortality of layer breeders was unaffected by FA dietary treatment. Clearly, supplemental folic acid is not required to maximize layer breeders fertility and hatchability.*
(Key words: folic acid, layer breeders, fertility, hatchability)

INTRODUCTION

The beneficial effects of folic acid (FA) on reproduction in humans, polytocous species and in some livestock animals have been well documented. Studies conducted in humans in the 1950s and 1960s led to the recognition of prenatal FA supplementation as a means to prevent pregnancy-induced megaloblastic anemia. The second major achievement with the use of FA occurred in the 1990s when periconceptional FA supplementation was found to reduce both the recurrence and occurrence of neural tube defects such as spina bifida, in children *(Tamura and Picciano,* 2006*)*. It has been experimentaly demonstrated that FA is critical for embryo survival and fetal development in rats *(Tagbo and Hill,* 1977*)*, hamsters *(Moiij et al.,* 1993*)* and guinea pigs *(Habibzadeh et al.,* 1986*)*. In sows supplemental FA has been associated with increases of about 10% in litter size at parturition *(Matte et al.,* 2006*)*. On the contrary, FA supplementation did not improve the reproductive performance of prolific and non-prolific ewes either in the estrous season or in the anestrous period *(Méthot et al.,* 2008).

Several *observations suggest* that poultry reproduction might be influenced by dietary supplements of FA *(Taylor,* 1947; *Sunde et al.,* 1950; *Robel,* 1993*)*. The current estimated requirement for FA for laying hens, based on experiments conducted in the

1950s, is between 0.21 to 0.31 mg/kg (*NRC,* 1994). Various contradictory results on the influence of supplementing diets with excess of FA on the egg folate concentrations have been reported in laying hens. *Dickson et al.* (2010) reported that the addition of 4 mg of FA/kg of laying hen diet lead to an increase of approximately 3-fold in egg folate concentration relative to a regular commercial egg. *Hebert et al*. (2005) supplemented as much as 128 mg of FA/kg of the laying hen diet but did not find any significant increase in egg folate concentration beyond the saturation level achieved at 4 mg of FA/kg in the diet. However, contrary to these observations, *House et al.* (2002) observed that egg folate concentrations increased above a plateau value when the level of FA in the diet was 32 mg/kg of diet. *Naber and Squires* (1993) found that the transfer efficiency of FA from diet to egg is very low. Considering the results obtained in the latter two studies we hypothesized that excess dietary FA might increase the amount of FA deposited in the egg and consequently resulting in improved reproductive performance success. Research on this topic is scarce. The goal of our study was, therefore, to assess the effect of two practical chicken diets, with various FA concentrations on the reproductive parameters of layer breeders.

MATERIAL AND METHODS

Experiment was conducted at a poultry research station (Biotechnical Faculty, University of Ljubljana, Slovenia) and the animal care and use protocol was approved by the Animal Welfare Council of the same Faculty. 1011 parent stock chickens (906 hens of dam line and 105 roosters of sire line) of Slovenian provenance Barred Prelux were selected at 35 wk of age, randomly allocated to control (n = 473 hens + 55 roosters) and FA-treated groups (n = 433 hens + 50 roosters), and raised in floor pens covered with wood shavings in an environmentally controlled facility. Each experimental group was replicated 2 times. The control group was fed with the basal diet, and the FA group was fed with the basal diet supplemented with 50 mg of crystalline FA/kg of diet (Farmalabor s.r.l., Milano, Italy). The basal diet consisted of antibiotic-free layer mash formulated to meet minimum nutrient requirement for layers established by *NRC* (1994) and included no crystalline FA or commercially produced 5-methyltetrahydrofolate. Composition and calculated nutrient content of the basal diet is given in *Table 1*. The basal diet contained 0.85 mg of total FA (from natural FA in feed ingredients) per kilogram of diet. Birds were fed with the experimental diets for a period of 4 wk (the first wk served as the adaptation period). Feed and water were available to permit ad libitum consumption. The photoperiod was fixed at 14h with illumination provided by fluorescent lights at an intensity range of 5 to 10 lux at chicken head height. Egg production and mortality were recorded daily. Feed consumption from all birds in each replicate was recorded on a weekly basis and the intake was calculated per bird per day. For the analyses of fertility, hatchability and body weight of newborn chicks, eggs were collected daily saved for hatching in a cool room and incubated 2 times at 10 d intervals. Before setting in the incubators eggs from the different treatments/replicates were labeled with a pen number, weighed and fumigated. A total of 11005 hatching eggs were used. The incubation was carried out in a single-stage electronically controlled setter, Petersime S168 (Petersime, Zulte, Belgium), at 37.8 °C and 60% RH. On day 18, the eggs were transferred to a hatcher, Petersime H168, with 37.2°C and 70% RH to complete incubation. After 21 d of incubation, chicks that had fully emerged from their shells were removed, weighed and their number was recorded. Unhatched eggs were broken, and classified by macroscopic examination as dead-in-shells or as infertile.

Dead-in-shells also included pipped eggs. Pipped eggs contained chicks that were not able to complete hatching successfully or were already dead. Percentage fertility and hatchability for each treatment group were calculated. Hatchability was calculated and expressed as a percentage of fertile and total eggs set. Daily egg production was calculated as percentage hen-day egg production. Data were subjected to ANOVA, using the PROC GLM procedure of SAS software (*SAS Institute Inc.,* 2004). All data in percentage form were transformed using arc-sine transformations prior to analysis. Pen constituted the experimental unit. The model tested the main effects of treatment, hatch time (two hatches - two ages of layer breeders), replication (pen) as well as the interaction terms using residual error. Because two-way interactions were not significant ($P>0.05$), data were analyzed for the main effects.

Values are expressed as least squares means (LSM) ± standard error of the mean (SE). Treatment means were separated using Tukey's test. Statistical significance was accepted at $P < 0.05$.

Table 1

Ingredient and calculated nutrient composition of standard layer basal diet

Ingredient	Percentage of ingredient
Maize (8.5% CP)	60.0
Soybean meal (49.5% CP)	17.0
Soybean oil	1.0
Sunflower meal (41.5% CP)	6.0
Maize gluten meal (60% CP)	1.0
Sugar beet molasses (10.1% CP)	3.0
Calcium carbonate	8.8
Calcium sodium phosphate	2.0
Mono-dicalcium phosphate	0.7
Sodium bicarbonate	0.2
Sodium chloride	0.2
Dietary supplements[1]	0.03
Calculated nutrient composition	
Metabolizable energy	2698.96 kcal / kg
Crude protein	16.2 %
Crude fibre	3.2 %
Crude fat	3.6 %
Crude ash	11.6 %
Lysine	0.75 %
Methionine	0.40 %
Calcium	3.50 %
Available phosphorus	0.50 %
Sodium	0.15 %
Folic acid	0.85 mg/kg

[1]Provided (per kg of diet): vitamin A, 10000 IU; vitamin D3, 2500 IU; 25 mg iron (from $FeSO_4 \times H_2O$); 6 mg copper (from $CuSO_4 \times 5H_2O$); 100 mg manganese (from MnO); 50 mg zinc (from $ZnSO_4 \times H_2O$); 0.3 mg cobalt (from $2CoCO_3 \times 3Co(OH)_2 \times H_2O$); 0.68 mg iodine (from KI); 0.15 mg selenium (from Na_2SeO_3); 5.4 mg butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT); 9.33 mg ethoxyquin; 1 mg **ethyl ester of ß-apo-8'-carotenic acid; 4 mg c**anthaxanthin; 10 mg lutein; 100 mg 6-phytase

RESULTS AND DISCUSSION

The supplementation of corn based diet with 50 mg crystalline FA did not affect egg weight before incubation (*Table 2*). These results agree with observations of *Hebert et al*. (2005) who researched two Leghorn strains of laying hens supplemented with 2, 4, 8, 16, 32, 64 and 128 mg/kg of FA and observed no difference in egg weight during a 3-wk experimental period. Similar results were also reported by *Tactacan et al*. (2012), who noted no effect on egg weight when Shaver White laying hens were fed diet supplemented with 10 or 100 mg of FA/kg of diet. Similar results were obtained by *Khalifah and Shahein* (2006), who determined that the inclusion of 0–32 mg FA/kg diet in the Baheij chicken strain did not affect the egg weight.

Table 2

The effects of folic acid (FA) supplementation of the diets of layer breeders on egg weight, one day old chick weight, hatchability, percentage of infertile eggs and dead-in-shells

Trait	Group	LSM ± SE	P-value
Egg weight (g)[1]	FA	61.32 ± 0.11	0.6486
	Control	61.25 ± 0.09	
Chick body weight at hatching (g)[2]	FA	40.62 ± 0.13	0.0223
	Control	40.19 ± 0.12	
Hatchability of total eggs (%)	FA	85.96 ± 0.56	0.6534
	Control	85.62 ± 0.51	
Hatchability of fertile eggs (%)	FA	89.16 ± 0.48	0.8084
	Control	89.00 ± 0.44	
Infertile eggs (%)	FA	3.58 ± 0.29	0.5707
	Control	3.80 ± 0.26	
Dead-in-shells (%)	FA	10.44 ± 0.46	0.8225
	Control	10.58 ± 0.41	
Hen-day egg production (%)	FA	88.98 ± 0.60	0.0352
	Control	90.84 ± 0.60	
Feed consumption (g/bird per day)	FA	136.16 ± 1.38	0.0001
	Control	150.11 ± 1.38	

[1] Number of eggs weighed and set in incubators: 4917 (FA group), 6088 (control group)
[2] Number of chicks hatched and weighed: 4218 (FA group), 5200 (control group)

Krishnan (2010) reported that the supplementation of 0, 2 and 4 ppm of dietary FA affected egg weight in 29-week-old Single Comb White Leghorn Bovan hens. The highest egg weight was observed at 0 ppm of FA supplementation compared to 2 and 4 ppm.

In the present study, supplementation of the maternal diet with FA increased BW at hatch by about 1% ($P<0.05$) compared with that of chicks from hens fed basal diet (*Table 2*). The reasons for this improvement are unclear. The fact that eggs from FA hens were not havier than eggs from control hens may indicate that the amount of water that was lost by diffusion through pores in the eggshell during the incubation process was higher in eggs from control groups in comparison with eggs from FA groups. However, in the present study, eggs were not weighed individually for the 0 to 18 d

incubational period to determine variation in egg weight loss among eggs. Data referring to chick weights are in agreement with the results reported in turkey hens by *Robel* (1993), who has shown that poult weights were increased when turkey hens received higher dietary FA (5.51 mg FA/kg of diet) and when eggs were injected with FA.

In the current experiment, the fertility and hatchability were not affected by the inclusion of FA in the diet (*Table 2*). The same outcome was observed in the studies with turkey breeders. *Schweigert et al.* (1947) did not observe any difference in the hatchability of turkey breeders when the FA content of the diet was increased from 0.42 mg of FA/kg to 2 mg of FA/kg. *Robel* (1993) reported that neither supplementing practical turkey breeder diets nor injecting 25-d embryonated eggs with FA improved hatchability.

In the analysis of nonhatched eggs, no differences in percentage of infertile and percentage of dead-in-shells were detected between treatments (*Table 2*). In this evaluation, nonhatched eggs were opened and classified through a macroscopic visual examination as infertile and consequently some eggs that were classified as infertile eggs may have been fertile eggs that contained a dead embryo.

The inclusion of FA in the diet reduced the rate of egg production and feed intake compared of hens fed the control diet (*Table 2*). Several studies have been conducted to examine the effects of supplemental dietary FA on the productive performance of the chickens and reported results are somewhat contradictory. *Tactacan et al.* (2012) showed that percentage hen-day egg production and feed consumption were similar in the laying hens fed 0, 10 or 100 mg of FA/kg of diet. Similarly, *Keshavarz* (2003), *Bunchasak and Kachana* (2009)*, Hebert et al.* (2011) and *Benkova et al.* (2009) were unable to improve egg production by supplementing practical diets for laying hens with FA. On the other hand *Krishnan* (2010) reported improved egg production with FA supplementation at higher levels (2 and 4 ppm) compared to no (0 ppm) supplementation. *House et al.* (2002) reported that the addition of supplemental FA in graded levels (0, 1, 2, 4, 8, 16, or 32 mg FA/kg) to laying hen diets did not have any effect on egg production. However, average daily feed consumption was higher for birds consuming the diets fortified with 32 mg/kg FA when compared to those consuming diets containing FA at lower levels (4 mg/kg; 8 mg/kg; 16 mg/kg), but not when compared to birds eating FA at 0 mg/kg. This result is to a certain degree in agreement with later study by *Krishnan* (2010), wherein addition of supplemental FA to laying hen diets had a marginal, but significant effect on feed intake.

Contrary to above mentioned reports, in the present experiment, the inclusion of FA to the diet reduced egg production and feed intake. It is well known that FA requirement is affected by many factors such as layer hen strain and age, stage of production, basal diet, FA level, form in which FA is fed, feed and management protocol, environmental factors (heat, light, moisture), etc (*House et al.,* 2002; *Krishnan,* 2010; *Hebert et al.,* 2011). Because of the different experimental materials and methods applied, it is very difficult to directly compare the results from other experiments with those of the current study. The reduced feed intake and consequently decreased egg production might have been the result of appetite depression or low palatibility of high levels of FA. However, further works are needed to test these hypotheses. The livability of males from sire line and females from dam line was not affected by the dietary treatment.

CONCLUSIONS

1. The administration of supplemental FA to layer breeders via dietary supplementation was effective in improving body weight of newborn chicks.
2. Egg production and feed intake were adversely affected by adding supplemental FA.
3. Folic acid at a concentration of 0.85 mg/kg was not limiting for fertility and hatchability in the basal diet as indicated by similar fertility and hatchability of birds that received the basal diet and the basal diet plus additional FA.

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Texture of dry cat foods and its relation to preference

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ABSTRACT

Structure analyzer devices were constructed for individual breaking and compression force measurement for dry cat foods. Three commercially available products with different flavors (chicken, lamb, tuna) of two manufacturers (premium and good quality) were examined. The self-developed devices were fitted to a computer-controlled structure test equipment. From a methodological point of view the different granule size and a curved granule surface cause uncertain results with the application of individual breaking device. However the cat food structure characterization with the compression measurement device was successful. The measured force values varied between 0.9 and 1.38 N/mm^2. According to the results of a parallel animal preference test the feed consumption values were remarkably different between feed types. Concerning the feed intake of the most and least preferred cat foods more than fivefold difference (75 vs. 420 g) was measured. With joint evaluation of the structural and preference results the hardest product proved to be the most favorable for the cats. Interestingly the compression force and also the feed intake sequence proved to be identical in case of the measured cat food products.
(Keywords: cat, pellet hardness, feed preference)

INTRODUCTION

Commercial cat foods can be classified as wet, semi-wet or dry products. Dry foods typically with less than 12 percentage of water content are available in extruded, granulated, farinaceous form or a kind of biscuits. Traditionally the physical quality of extrudes can be characterized by the hardness and the structural stability which parameters have an outstanding importance in feed industry. According the results of *Tran et al*. (2011) the temperature, the duration of drying time, the particle size and the moisture content have remarkable effects on hardness and structural stability. These physical parameters are important not only from animal but also from packaging and transporting point of view as the breakable granules lead to quality problems.

The so-called plaque development of dogs and cats can be considered as a serious health problem. The tartar coated teeth provides excellent conditions for microorganism colonization causing inflammation, unpleasant breath and as a final result loosing of the teeth. The proper chewing helps to clean the teeth especially in wild animals getting more natural feed than our pet animals. However commercial pet foods are available which were developed considering the recognized correlation within pellet hardness and

the level of plaque formation (*Cupp et al*., 1999). The relationship of feed hardness and the occurrence of certain diseases – or more general the life quality were examined by *Bailoni and Cechiaro* (2005) in case of old dogs.

The producers identify the palatability as a measurable parameter of feed preference and feeding behavior. These measures are determined by the sense of taste, aroma, shape, particle size and structural characteristics (*Tran et al*., 2008). The feed preference of cat is mostly depending on the feed's smell which can be affected by microbiological contamination, rancidity, aroma distortion or in some cases structural degradation of the product (*Deffenbaugh*, 2007). It is well known that the hardness of feed particles have remarkable effects on feed preference of *animals in general* (*Skoch et al*., 1983). *According to the experiment of Trivedi* and Benning (1999) the cat rejects feed containing sharp particles which could cause injuries within the mouth or the stomach of the animal.

Aims: Method development and measuring device construction for dry pet food structure characterization based on the use of a universal texture analyzer. We also aimed to compare the animal acceptance and the structural characteristics of commercial cat food samples.

MATERIAL AND METHOD

The structural studies were carried out in the Laboratory of Kaposvár University Institute of Food and Agricultural Product Qualification by a computer-controlled structure analyzer type: ZwickRoell Z005. For the measurements self-developed devices were constructed considering the work of *Thomas and van der Poel* (1996) as basis. Two types of measurements (individual breaking and compression) were performed. The force on the measuring cell and the maximum compression force per unit area (N/mm^2) were continuously recorded with testXpert V11.0. software during the snap or the volume changes of the sample, which is directly related to the energy needed by the chewing process of the animals.

In the course of the methodological studies, we investigated the hardness of the commercially available dry cat foods. Three different flavored products (chicken-A, lamb-B, tuna-C) of two manufacturers in two different quality categories (premium-1 and good-2) were compared during our study.

The samples used in the structural analysis, were also independently analyzed by preference tests at Szent István University, Faculty of Veterinary Science, Department of Animal Breeding, Nutrition and Laboratory Animal Science. Based on sample selection and the implemented procedure, the cats could choose between three different feeds **(**chicken, lamb, tuna**)** from the same manufacturers per trial. These choices came from two (premium, good) different manufacturers. The 8 cats were kept in individual cages and were feed *ad libitum*. The feed consumption was recorded for 8 consecutive days.

The calculated average values of the recorded maximum shear force per unit area (N/mm^2) measured during the experiments were tabulated and graphically presented with Microsoft Excel 2010. For the statistical analysis (One-Way ANOVA), IBM SPSS Statistics 20 software was used. In order to compare the treatment means, Tukey test was applied ($P=\geq 0.05$) and finally, correlation analysis was performed to calculate the relationship between the studied variables.

RESULTS AND DISCUSSION

Device development

For the individual breaking of the food grains we designed a special, stainless steel pressure piston, with a diameter of 10 mm and round shaped, horizontal surface that exerted the pressure on the sample (*Picture 1*). The sample was placed on a horizontal, circular shaped appurtenance; an anvil with a diameter of 20 mm, which was approached by the moving piston with 50 mm/min speed. The distance between the anvil and the pressure piston was 16 mm in the starting position. The piston moved a distance of 14 mm during the measurement, causing the food sample to be tested to collapse under the generated pressure. For a given product, a set of 50 food grains were measured. Prior to the experiments, it was necessary to define the average cross section for all products in order to ensure the accuracy of the measured shear force by the instrument, since the use of incorrect cross section values could lead to the bias of the measured results and therefore an erroneous interpretation of them.

A 50 mm high and 50 mm inner diameter, stainless steel cylinder was prepared to perform the compression measurements (*Picture 2*). Inside the above specified cylinder, there was a solid piston of stainless steel (height: 12 mm, diameter: 44 mm) which was used for the compression. During this phase of the procedure, the piston compressed the samples only to 80% of original volume. The piston speed was 50 mm/min. The measurements were repeated 15 times.

Picture 1. Breaking device

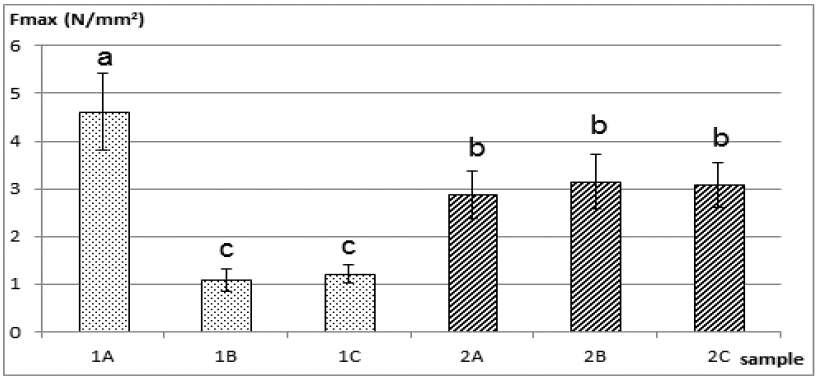
Picture 2. Compression device

Individual breaking of the granules

Remarkable differences were determined within the maximum breaking force values of the measured pet foods (*Figure 1*). The samples of the first producer can be divided into two well separated groups with significantly different force resistance. In case of the second brand the force measurement resulted similar values independently from the composition of the sample. Interestingly feeds with identical ingredient contents shows remarkably different structural characteristics presumable due to their manufacturing technology.

Figure 1

The maximum force required to individual breaking of feeds



Where: 1-premium, 2-good; A-chicken, B-lamb, C-tuna

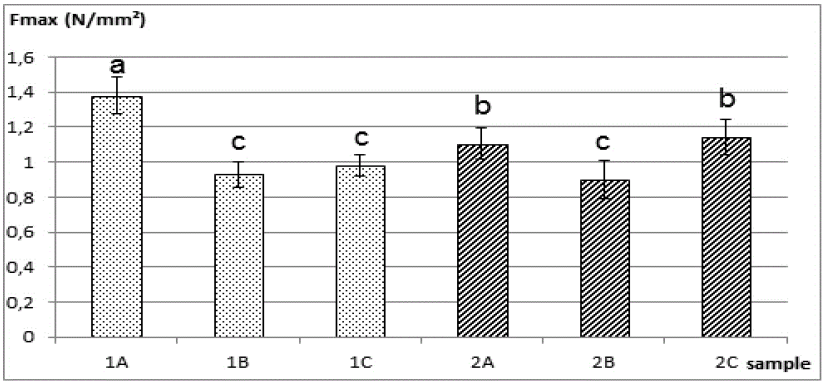
It is noteworthy that in case of a product with different granule sizes this approximation is not applicable. Further methodological problem arises if the granule surface is remarkably curved causing a so-called "two-step" structural degradation of the sample.

Compression measurement

During the course of methodological experiments the optimal sample volume was determined based on the maximum force value needs for the 80 percentage compression. According to our preliminary results 25 cm³ of sample volume proved to be adequate for the further measurements. It should be mentioned that particle size and shape have remarkable effects on the extent of compression. However in the present study this effect was negligible due to the similarity in size and shape of the examined samples.

Figure 2

Results of the compression measurement



Where: 1-premium, 2-good; A-chicken, B-lamb, C-tuna

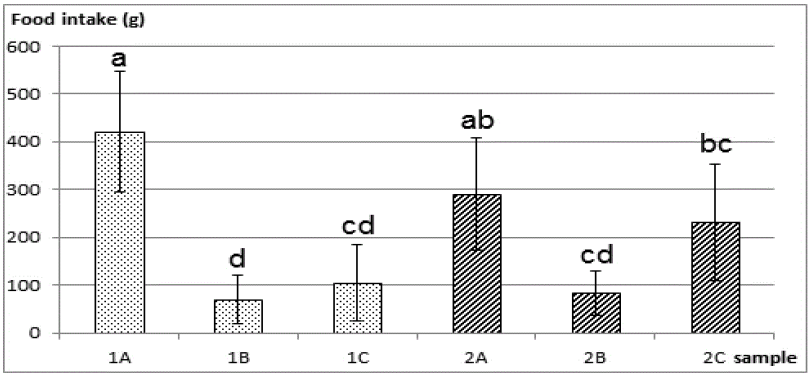
According the compression results the 1A samples proved to be the hardest among the six examined samples similarly to the results of the individual breaking measurement (*Figure 2*). There were statistically proven differences between the compression values of the samples. Based on the lower standard deviation values of the compression measurements this method seems to be more reliable than individual breaking. It should be noted that the different granule shapes and sizes effect the compression rate which modify the results remarkably.

Preference test

The average feed consumption values were remarkably different between the sample-groups with similar feed composition. The difference within the most and least preferred cat foods were more than five-fold in food intake (*Figure 3*). On the other hand it is obvious that the taste characteristics have a significant effect on the preference order, too.

Figure 3

Average feed consumption



Where: 1-premium, 2-good; A-chicken, B-lamb, C-tuna

According the results the hardest 1A product proved to be the most favorable cat food. Interestingly the order of the compression force needed (*Figure 2*) is similar to the feed intake order of the different cat food products. Finally a strong correlation (r = 0.97) was obtained between these structural and preference data.

CONCLUSIONS

The structure analyzer device development was successful. From methodological point of view the compression equipment was useful in structural characterization of the dry pet food samples in a stable and reproducible manner. Using the method, remarkable structural variations were described within the food samples with different composition provided by different producers.

The hardness of the dry cat food granules has a substantial effect on their preference. Regarding to the composition and structure interactions further examinations are needed to describe the interrelations in detail.

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Application of BovineSNP50 genotyping array in variability assessment in Pinzgau bulls

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ABSTRACT

The aim of this study was to evaluate the level of SNP polymorphisms and describe the basic characteristic of the analysed population genotyped using the BovineSNP50 genotyping array, which has lot of applications in cattle such as genome association studies, genetic prediction of breeding values, estimation of genetic diversity and population genetic parameters and investigation of genetic relationships among cattle breeds. In total 19 purebred Pinzgau bulls were successfully genotyped with Illumina BovineSNP50 BeadChip (98.96% of SNPs) with call rate 0.995. Genotyping results from 54,906 SNPs revealed that 43,120 SNPs (78.96%) were polymorphic with average minor allele frequency 0.273±0.133. Within 43,120 SNPs genotyped, 98.19% were autosomal, with 776 polymorphic SNP on chromosome X and only one on chromosome Y. The average values of observed and expected heterozygosity across polymorphic loci were 0.375±0.157 and 0.362±126, respectively. Sufficient proportion of heterozygotes indicated the value of F_{IS} (0.037±0.031). Genomic data obtained for purebred Pinzgau bulls from the BovineSNP50 chip can be in further applied for evaluation of genetic diversity in Pinzgau breed as endangered population of cattle in Slovak republic.
(Keywords: cattle, MAF, polymorphism, SNP50 chip)

INTRODUCTION

Genomics is currently being utilized for genetic evaluations, parentage verification and screening for lethal recessives, congenital disorders and other mutations with large effects on performance in cattle populations (*Mullen et al.*, 2013). In recent years the SNP array has been developed, based on the discovery of numerous SNPs through genome sequencing. This technology has been evaluated as a new high-throughput genotyping technology since it enables simultaneous detection of the loci of a large number of SNPs (*Suekawa et al.*, 2010). The availability of many thousands of single nucleotide polymorphism (SNP) markers distributed across the genome has led to a new approach for genetic studies and applications (*Van Tassel et al.*, 2008). SNPs are currently used in genome wide-association studies (*Bolormaa et al.*, 2011; *Schopen et al.*, 2011); in genetic prediction of breeding values (*Meuwissen and Goddard*, 2010) or for estimation of genetic diversity and population genetic parameters (*Engelsma et al.*, 2012). Among livestock species, this technology has been applied most successfully in cattle, because factors such as evolutionary history, genetic structure, economics, etc. make cattle particularly suitable for the application of genome assisted selection

(*Nicolazzi et al.*, 2014). Genotyping of cattle using SNP array has become common practice in dairy cattle breeding programs applying genomic selection (*Mulder et al.*, 2012).

A number of SNP chips from Illumina and Affymetrix are available for cattle. These include 3K, 7K, 15K, 25K, 50K and more recently 800K from Illumina, and 650K and 3 million SNP panels from Affymetrix. In addition next generation sequencing technologies for low-cost sequencing of whole genomes are now available (*Khatkar et al.*, 2012). The release of the Illumina BovineSNP50 BeadChip in late 2007 has drawn attention from cattle breeders around the world to develop breeding programs that leverage association of these single nucleotide polymorphism (SNP) with economically important quantitative trait loci (QTL) (*Lu*, 2012). Moreover the availability of large numbers of SNP markers has resulted in new opportunities to estimate genetic diversity in more detail, and to improve prioritization of animals for conservation of genetic diversity. Conservation of genetic diversity in livestock breeds is important since it is, both within and between breeds, under threat. Over the last decades, genetic diversity of livestock populations had been alternatively measured using pedigree information or microsatellite data when genealogy is not available. Currently, the availability of high-density SNP chips has opened up new opportunities to evaluate genetic diversity based on genetic markers. Up to now, conservation decisions for gene banks were often based on pedigree information, while the use of high-dense markers may give a more detailed picture of the diversity across the genome (*Engelsma et al.*, 2011).

The objective of this study was to evaluate the level of SNP polymorphisms and describe the basic characteristic of the population genotyped, using the BovineSNP50 BeadChip.

MATERIAL AND METHODS

Breed description

The Pinzgau cattle is traditional dual purpose type breed of mountainous areas of Slovakia, introduced in 18th century (*Kasarda et al.*, 2008). It was imported from region of the Austrian Alps and populations' breeding in Slovakia is from its constitution over connected (*Pšenica*, 1990). The first imports of Pinzgau purebred animals were organized long time ago before 1894 when system of cattle recording has started on territory of Slovakia. The size of breed was improving and in 1958 it was officially accepted as Slovak Pinzgau (P) breed (*Kadlečík et al.*, 2013). In 1970 re-building of purpose and breed type of cattle started. In this period several breeding bulls were imported, from which 24% were of Pinzgau origin (*Pšenica and Tretinova*, 1998). Highest number of cows bred was 162 127 in 1970 (*Pšenica*, 1998). From 1970 to 1992 in pedigrees of Pinzgau cattle were introduced seven breeds, almost 50% Pinzgau bulls imported from Austria and 34% from Slovakia. From 1994 Pinzgau cattle in Slovakia is registered by FAO as endangered. After 1990 considerable decrease of Pinzgau population size was observed in Slovakia (*Kadlečík et al.*, 2011).

Sample collection and DNA genotyping

For genomic evaluation 19 AI Pinzgau proven sires were selected. Selection criterions were as follows: sires present on AI stations with reliability of breeding value over 65% and born from 1998 to 2006 in Slovakia. Except one, all sires were born in Slovakia, only Nero from lineage Nusil bought as young sire in Austria. Five sires had father of Slovak origin (26%) and only two grandfather (10%). All sires represent 13 lineages of

dual purpose Pinzgau cattle. Most frequent lineage was Nobel represented by 4 sires, followed by lineage Nusil by 3 sires and Origin of foreign sires in pedigrees was Austrian. In long term scope Austrian sires were often used for mating of sire dams. Genomic DNA for each of the 19 bulls semen samples was genotyped at a commercial lab using an Illumina BovineSNP50 Genotyping BeadChip.

Statistical analyses

Quality controls and computation for SNP data was performed with PLINK (*Purcell et al.*, 2007). The following quality control criteria (filters) were used to remove from further analysis any SNPs with less than 95% call rate, and SNPs with less than 0.05 MAF. SNP were tested for HWE ($P<0.001$) to identify possible typing error. Samples with more than 10% missing genotypes were removed from the study. Heterozygosities and fixation index (F_{IS}) for analyzed population were estimated.

RESULTS AND DISCUSSION

In this study was evaluated application of the BovineSNP50 genotyping array in the Slovak Pinzgau cattle population. Nineteen purebred Pinzgau bulls were genotyped with Illumina BovineSNP50 BeadChip. All samples were successfully genotyped (98.96% of SNPs). Total call rate (99.45%) was comparable with average rate reported by *Cooper et al.* (2013) across 3 dairy cattle breeds and *Mullen et al.* (2013) across beef and dairy cattle originating in *Bos taurus*. Similarly high values of call rates (>98%) were reported also for cattle breeds originating from *Bos indicus* (*Qwabe et al.*, 2013; *Neves et al.*, 2014). These results validated that the BovineSNP50 BeadChip is important genomic tool for different bovine breeds, which can be subsequently used for multiple application in GWAS, genomic selection or evaluation of genetic relationship between breeds.

Table 1

Minor allele frequencies

Frequency	Autosomes		Sex chromosomes	
	# loci	**%**	**# loci**	**%**
0.0–0.1	4713	11.131	83	10.682
0.1–0.2	9273	21.900	137	17.632
0.2–0.3	9749	23.024	199	25.611
0.3–0.4	9832	23.220	185	23.810
0.4–0.5	8776	20.726	173	22.265
Total # of polymorphic loci	*42343*		*777*	

Genotyping results from 54,906 SNPs revealed that 43,120 SNPs (78.96%) were polymorphic with minor allele frequency greater than 0.05 (*Table 1*). The level of polymorphic SNPs in the present study was higher than in previously reported study for cattle (*Mai et al.*, 2010; *Hulsegge et al.*, 2013). Minor allele frequency (MAF) is widely used to describe the genetic variability of two-allele SNPs, and refers to frequency of the least common SNP allele (*Haynes et al.*, 2012). Within 43,120 SNPs genotyped, 98.19% were autosomal, with 776 polymorphic SNP on chromosome X and only one on chromosome Y. The average MAFs across loci on autosomes were 0.273±0.133 and sex

chromosomes 0.272±0.132 with minimum value 0.053. The average values of MAF depend of cattle breeds. Considerable variations in MAF between breeds reported *Matukamelli et al.* (2009), who observed higher proportions of polymorphic SNP in Holstein and Angus cattle in comparison with African N'Dama and Sheko breeds. The average values of observed and expected heterozygosity across polymorphic loci were 0.375±0.157 and 0.362±126, respectively. Sufficient proportion of heterozygotes indicated the value of F_{IS} (0.037±0.031).

CONCLUSIONS

Molecular genetic tools are in present preferably used in description of genetic composition of populations. Statistical analysis of molecularly based data gives deeper insight into genetic variability and helps in evaluation and monitoring of populations´ diversity. Use of the Illumina BovineSNP50k BeadChip is standard tool in studies such as genetic diversity, genomic breeding values estimation, evaluation of population structure and genetic distances. Further research will be oriented on evaluation of diversity issues of the Pinzgau population.

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Effect of the fat mass and obesity associated (FTO) gene polymorphism on carcass traits in pigs

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ABSTRACT

The aim of this study was to evaluate the effect of FTO single nucleotide polymorphism (g.276T>G) on carcass traits in Large White pigs. In several studies have been suggested that the FTO gene influenced significantly regulation of energy balance and feed intake in pigs and therefore can be considered as genetic marker for their production traits. We used 150 boars (73) and sows (77) from Large White breed. Genotyping of animals was carried out by PCR-RFLP method with using restriction enzyme TaiI. We identified three genotypes TT (20.66%), TG (66.67%) and GG (12.67%). Associations of FTO gene polymorphism with back fat thickness, lean meat percentage, thigh percentage and MLT area were analyzed. The results of the statistical analyses did not confirmed the effect of the FTO mutation (g.276T>G) on selected carcass traits in analyzed population of Large White pigs.
(Keywords: carcass traits, FTO, SNP g.276T>G, pig)

INTRODUCTION

Knowledge on the genetic background of fat tissue accumulation is important in livestock production. Several fatness traits are considered in pig breeding improvement, most frequently back fat thickness and intramuscular fat content. The fatness traits are related to meat quality or fattening efficiency (*Switonsky et al.*, 2010). The breeding goals in modern pig breeds often include decreased back fat, abdominal fat weight and optimal level of intramuscular fat. Therefore, the knowledge on the contribution of the pig FTO gene to fat accumulation variability is important for meat production (*Szydlowski et al.*, 2012).

The amino acid sequence of the FTO gene showed high conservation among human, pig and other important domestic animals. The FTO gene is highly expressed in the hypothalamic-pituitary-adrenal axis, suggesting this gene may participate in the central control of energy homeostasis or in the development of fat tissue (*Zhang et al.*, 2009). The pig FTO gene was mapped to SSC6 (*Fontanesi et al.*, 2009). Several studies reported relationship between phenotypes and genetic variants of FTO gene in different breeds of pigs. *Fan et al.* (2010) found in Yorkshire pig experimental population significant associations between SNPs in FTO gene and residual feed intake. *Szydlovsky et al.* (2012) reported for FTO gene multiple significant associations with back fat thickness, abdominal fat weight and lean meat content in Polish Landrace pigs and therefore can be associated with fatness traits in purebred pigs selected for low fatness. Results of *Dvořáková et al.* (2012) study show that in commercial pig populations FTO

influences back fat depth. *Zhang et al.* (2009) found in 6 Chinese native pigs breeds population significant association only between FTO gene and intramuscular fat content. The association analyses of *Fontanesi et al.* (2010) confirmed the effect of the FTO mutation on obesity-related traits (visible intermuscular fat, back fat thickness and lean cuts) in the Italian Duroc pigs.

The aim of this study was to analyse the effect of FTO g.276T>G single nucleotide polymorphism on carcass traits in Large White pigs.

MATERIAL AND METHODS

Animals used in the study included boars (n=73) and sows (n=77) of Large White pigs from the Experimental Centre of Farm Animals, Department of Animal Husbandry, Slovak University of Agriculture in Nitra.

Genomic DNA was extracted from blood samples using protocol according to *Miller et al.* (1988). Concentrations of DNA were estimated by spectrophotometer measurement by the optical density at wave length of 260 nm. For genotyping of animals was used PCR-RFLP method. A 397 bp fragment of intron 4 in porcine FTO gene was amplified by PCR using forward and reverse primers according to *Fontanesi et al.* (2010). The polymerase chain reaction was performed in a 25 µl reaction mixtures, containing: 10 x PCR reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 4 pM of each primer, 1 U Tag DNA polymerase (Fermentas), 50 ng genomic DNA. Thermal cycling conditions included: an initial denaturation step at 95 °C for 2 min, followed by 40 cycles of 95 °C for 30 sec, 64 °C for 40 sec, 68 °C for 50 sec and a final extension at 68 °C for 7 min. PCR products of FTO gene were subsequently digested with 1 µl of FastDigest *Tai*I (Fermentas) restriction enzyme at 37 °C in time 15 min and separated by horizontal electrophoresis in 3% agarose gels in 0.5 x TBE (130 V for 40 min) stained with GelRed (Biotium) prior to visualization under UV light.

The allele and genotype frequencies of FTO gene were estimated by direct counting and examined for deviation from Hardy – Weinberg equilibrium using Chi-square (χ^2) test. The analyzed carcass traits – back fat thickness (BFT), lean meat percentage (LM), thigh percentage (TP) and MLT area were measured by standard technical norm STN 466164. Estimates of the effects were tested by t-test for significant deviation from zero. Association analysis of the SNP g.276T>G in FTO gene was performed using GLM (General Linear Model) procedure of SAS Enterprise Guide 4.2 software (*SAS Institute Inc.*, 2009) with the following model:

$$Y_{ijk} = \mu + G_i + S_j + W_k + e_{ijk}$$

where: Y_{ijk} – dependent variable (analyzed carcass traits), μ – the general mean, G_i – genotype, S_j – sex, W_k – live weight (kg) as covariate, e_{ijk} – random error.

RESULTS AND DISCUSSION

Three genotypes were identified in the analyzed group of pigs, TT (n=31), TG (n=100) and GG (n=19). Allele T showed higher frequency than allele G (0.54 vs. 0.46). The population was in Hardy-Weinberg equilibrium (P>0.05). Allele and genotype frequencies are presented in *Table 1*. These results are similar to the reported by *Fontanesi et al.* (2009) and *Dvořaková et al.* (2012), where the g.276T allele was the predominant in Large White, Duroc, Landrace, Hampshire and Pietrain pigs. *Fontanesi et al.* (2010) also confirmed lower occurrence of g.276G allele in Italian Duroc and

commercial pig populations. *Table 2* and *3* shows average values of analyzed carcass traits in relation to specific genotype in analyzed population of pigs.

Table 1

Alleles and genotypes frequencies of FTO g.276T>G marker in pigs

Genotype frequency			Allele frequency		χ^2 test
g.276TT	**g.276TG**	**g.276GG**	**g.276T**	**g.276G**	1.23^{-}
20.66	66.67	12.67	0.54 ± 0.029	0.46 ± 0.029	

P>0.05

The observed associations of individual genotypes of FTO gene with the values of analyzed carcass traits are presented in *Table 3*. In the group of evaluated pigs statistical analyses shows only non-significant associations between the variability of back fat thickness, lean meat percentage, thigh percentage and MLT area and different FTO g.276T>G genotypes. Statistically significant effect was found only for sex (P<0.0001) and live weight (P<0.01). The highest value of BFT was found for heterozygous animals. In group of GG homozygote was observed the best value of any others evaluated carcass traits, but differences were low and non-significant (P>0.05).

Table 2

Basic statistical variation measurements carcass traits in pigs

	n	**mean**	**SD**	**min**	**max**
BFT (mm)	150	18.86	4.39	8.67	28.67
LM (%)	150	53.82	2.62	45.14	63.23
TP (%)	150	21.89	1.50	17.59	28.82
MLT area (cm^2)	150	41.79	5.62	26.90	62.50

Table 3

The effect of FTO g.276T>G genotypes on carcass traits in pigs

Trait	**Genotype**								
	g.276TT			**g.276TG**			**g.276GG**		
	n	**mean**	**SD**	**n**	**mean**	**SD**	**n**	**mean**	**SD**
BFT (mm)	31	18.82	3.96	100	19.06	4.54	19	17.85	4.38
LM (%)	31	53.86	2.61	100	53.76	2.78	19	54.08	1.69
TP (%)	31	22.12	1.54	100	21.77	1.55	19	22.19	1.10
MLT area (cm^2)	31	42.82	5.71	100	41.09	5.59	19	43.74	5.21

P>0.05

Important QTL for carcass and meat quality traits localized on SSC6 have been reported in many studies (*Fan et al.*, 2009; *Zhang et al.*, 2009; *Fontanesi and Russo*, 2012;

Szydlovsky et al., 2012), but the effect of g.276T>G single nucleotide polymorphisms in FTO gene was assessed only in a few studies. *Fontanesi et al.* (2009) have identified this FTO polymorphism in intron 4 in associations with intermuscular fat deposition in the Duroc breed and with feed conversion rate in Italian Large White pigs. These results have been confirmed in subsequent analyses on Italian Duroc ($P<0.01$) and commercial pig populations ($P<0.05$) (*Fontanesi et al.*, 2010).

CONCLUSIONS

The SNP g.276T>G in FTO gene was analysed for associations with carcass traits in Large White pigs. In our study it doesn't confirmed the findings of other authors on the effect of FTO gene on production traits in pigs. The associations with analyzed carcass traits showed only statistically non-significant results, but different studies between FTO polymorphisms and production traits indicated that this gene has a major role in the variation of fatness traits. One of the causes can be genetic background effect and limited population size of evaluated animals. To be able to consider FTO gene as genetic marker in assisted selection programs in commercial as well as purebred pig population it is needed to confirm its effect on economically important traits.

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Genetic polymorphism of Pit-1 gene associated with milk production traits in Holstein cattle

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ABSTRACT

The aim of this study was to evaluate the effect of Pit-1 gene polymorphism on long-life milk production traits in the selected population of Holstein cattle. Biological material was obtained from hair roots of 89 Holstein cows originating from the farm with high milk production in Slovak Republic. We use the PCR – RFLP method to detect Pit-1 gene polymorphism (restriction enzyme HinfI). In cows population we identified the presence of all three genotypes: AA genotype (260 bp) of 5 cows, genotype AB (260 bp, 190 bp, 70 bp) of 23 cows and BB genotype (190 bp, 70 bp) of 61 cows. The highest frequency had homozygous BB genotype (69%). The frequency of occurance of allele B (0.81 ± 0.03) was higher than the frequency of allele A (0.19 ± 0.03). The level of locus polymorphism in the analyzed population was median (PIC=0.2564). We observed unbalanced activity of alleles (N_e=1.43) and a higher ratio of homozygous individuals, which caused decrease of expected heterozygosity (H_e=0.31). Evaluation of Pit-1/HinfI genotype effect on milk production traits was carried out with linear models (GLM procedure). Based on the selected effect we were able to estimate the variability of analyzed traits on 79%. The Pit-1/HinfI genotype affected the variability of milk, protein and fat yield in long-life production only non-significant (P>0.05).
(Keywords: cattle, milk traits, Pit-1, polymorphism)

INTRODUCTION

Studies conducted in recent past have successfully determined the genotypic profiles at many economically important candidate gene loci like κ-casein, β-lactoglobulin, growth hormone, butyrophyllin, STAT-5a, prolactin, pituitary specific transcription factor etc. in several cattle breeds (*Mukesh et al.*, 2008).

Pituitary transcription factor (Pit-1) has been shown to be a positive regulatory factor of growth hormone (GH), thyrotrophin β-subunit (TSH- β), and prolactin (PRL) in the mammalian pituitary (*Doosti et al.*, 2011). Genetic and biochemical analysis indicated the main role of Pit-1 in the cell stimulation of growth hormone during gene transcription (*Aytekin and Boztepe*, 2013). The inhibition of Pit-1 synthesis leads to a marked decrease in expression of PRL and GH to a dramatic decrease in proliferation of cell lines producing PRL and GH (*Beigi et al.*, 2010; *Heidari et al.*, 2012; *Selvaggi and Dario*, 2011). Expression of Pit-1 gene is superior to growth hormone and prolactin genes (somathotropic and lactothropic cells) and also to expression of hormone specific activators of these cells (*Scully et al.,* 2000). In mammals, Pit-1 has three different splicing variants, the major type, Pit-1α, and two other splicing variants, Pit-1β and Pit-1T (*Theil et al.*, 1989). All of the splicing variants are biologically active. Pit-1 variants

act differentially on the promoters of target genes. Pit-1α strongly activates Pit-1α and Pit-1β promoters (*Tanaka et al.*, 1999). Pit-1β has a 26-amino acid insert in the transactivation domain because of alternative splicing of the Pit-1 gene transcript at the end of intron one. Consequently it lost the ability to activate the PRL and Pit-1 promoters and preferentially activates the GH gene promoter. Pit-1T contains a 14-amino acid insert in the transactivation domain because of an alternate 3' splice acceptor site and was found to be expressed in thyrotroph-derived cells and stimulates only TSH-β expression (*Ferry et al.*, 2005; *Zhao*, 2002). Bovine Pit-1 as 129 amino acid protein is member of DNA-binding POU family of homeo-domain transcription factor. This has been sublocalized to the centromeric region of bovine chromosome 1, located midway between TGLA57 and RM95 (*Moody et al.*, 1995).

In Pit-1 gene have been until now indentified various mutations, which caused genetic disorder in regulation of growth hormone and prolactin. Because they are necessary for development of mammary gland and consequently milk production gene encoding Pit-1 have a great potential as genetic marker for evaluation of milk production traits. The aim of this study was to evaluate the effect of Pit-1 gene polymorphism on long-life milk production traits in the selected population of Holstein cows.

MATERIAL AND METHODS

In this study were analyzed a total of 89 biological samples obtained from selected Holstein cows originating from the farm with best milk production in Slovak Republic. Genomic DNA for molecular – genetic analysis was extracted from hair roots according to *Gábor* (2009) and concentration was estimated by spectrophotometer measuring of the optical density at wave length of 260 nm. Following identification of Pit-1 polymorphism/genotypes was performed by PCR-RFLP method with using primers (FOR: 5´ -ACT CGC TAT TAC ACA ATA GGA GAG CCT- 3´, REV: 5´-TCC TGC CAA CTC CTC ACC TCC C - 3´) according *Ozdemir* (2012). A genotyping of Pit-1 allelic variants was carried out by digestion of 260 bp PCR products and *Hin*fI restriction enzyme. Results from PCR amplifications and digestion of PCR products were analyzed by horizontal electrophoresis in 3% agarose gels in 0.5 x TBE (130 V for 40 min) (*Broody and Kern*, 2004) stained with GelRed (Biotium) prior to visualization under UV light.

Frequency of Pit-1 alleles and genotypes for the entire population were estimated by direct counting and the differences of the observed and expected frequencies of genotypes were tested using Chi-square (χ^2) analysis. Genetic indices of populations, including observed and expected gene heterozygosity (H_e), homozygosity (H_o) and effective allele numbers (N_e) were performed by Popgene32 software version 1.3 (*Yeh et al.*, 2000). Moreover, polymorphism information content (PIC) was calculated according to *Botstein et al.* (1980).

Associations of the Pit-1 genotypes with milk production traits were determined by analyses of quantitative traits. Statistical analysis was performed using SAS Enterprise Guide 4.2 software (*SAS Institute Inc.*, 2009) and significance of differences based on genotypes effect of production and reproduction traits were tested by following general linear models:

$$Y_{ijklmn}{}^{1,2,3} = G_i + BT_j + S_k + \mathbf{A_l} + L_m + DL_n + e_{ijklmn}$$

where: $Y_{ijklmn}{}^{1,2,3}$ – milk, protein and fat yield, G_i – effect of Pit-1/HinfI genotype, BT_j – effect of breed type, S_k – effect of sire, A_l – effect of age at first calving, L_m – effect of number of lactation, DL_n – effect of days of lactation, e_{ijklmn} – random error.

RESULTS AND DISCUSSION

The Pit-1 gene located on bovine chromosome 1 consists of 6 exons. In the 6 exon of bovine Pit-1 gene the restriction fragment length polymorphism using restriction enzyme *HinfI* was detected (*Moody et al.*, 1995). *Table 1* summarised alleles and genotypes frequencies of Pit-1 gene polymorphism (called HinfI) in the analyzed populations of Holstein cows. The highest frequencies were observed for homozygous BB genotype, and lowest for AA genotype. Analyzed population was in Hardy-Weinberg equilibrium (P>0.05). High frequency of BB genotype was reflected also in observed frequency of B allele, which was predominant. Basic genetic indices of population are presented in *Table 1*. Number of heterozygous animals (26%) was transferred to the value of expected heterozygosity. Analyzed locus showed based on the expected heterozygosity only median level of polymorphic information content. The effectiveness of loci allele impact in population has been expressed by effective allele numbers. Effective allele number demonstrated unbalanced activity of alleles in a analyzed population of cows.

Table 1

Distribution of SNP Pit-1/HinfI alleles and genotypes in Holstein cows

Genotype frequency			Allele frequency		χ^2 test	H_o	H_e	N_e	PIC
Pit-1/HinfI^{AA}	Pit-1/HinfI^{AB}	Pit-1/HinfI^{BB}	Pit-1/HinfI^{A}	Pit-1/HinfI^{B}	1.85^{-}	*0.69*	*0.31*	1.43	0.26
0.05	0.26	0.69	0.19±0.03	0.81±0.03					

P<0.05

Table 2 shows average values of analyzed long-life milk production traits in whole population. Overall was found in cows population maximum of 5 completed lactations. The largest number of cows had completed only 1. lactation (38.20%). Number of completed lactation, sire, breed type, days of lactation and age at first calving were included as fixed effects in linear model (GLM procedure) for evaluation of Pit-1/HinfI genotypes influence on milk production traits. *Table 3* shows average values of analyzed traits in relation to specific Pit-1/HinfI genotype and significance of selected fixed effect. The Pit-1/HinfI genotype affected the variability of analyzed traits only non-significant (P>0.05). Based on the selected effect we were able to estimate the variability of traits on 79%. Statistically significant effect was found only for sire (P<0.01), age at first calving (P<0.05), number of completed lactation (P<0.01) and days of lactation (P<0.0001). In cows population was observed average age at first calving 743.98 ± 91.89 and days of lactation 310.03 ± 113.04.

Table 2

Basic statistical variation measurements of milk production traits

	n	mean	SD	min	max
Milk yield (kg)	89	9977.54	3885.60	564.00	21584.00
Fat yield (kg)	89	356.67	132.67	27.00	772.00
Protein yield (kg)	89	320.87	125.24	23.00	656.00

Table 3

The association of Pit-1 gene polymorphism with milk production traits

	Pit-1/HinfI^{AA}			Pit-1/HinfI^{AB}			Pit-1/HinfI^{BB}			Factors
	n	mean	SD	n	mean	SD	n	mean	SD	Breed type Sire** Age at 1.calving* Number of lactation** Days of lactation***
MY(kg)	5	10324.00	1293.69	23	9457.49	3767.20	61	10132.05	3959.86	
FY (kg)	5	409.33	37.11	23	338.37	135.77	61	361.23	132.86	
PY (kg)	5	359.33	37.74	23	303.76	125.35	61	325.36	126.37	

MY – Milk yielk, FY – Fat yield, PY – Protein yield, *P<0.05, **P<0.01, ***P<0.0001

The development and function of mammary gland is mainly controlled by growth hormone and prolactin, two protein hormones secreted by the anterior pituitary gland. Their synthesis is under regulatory influence of pituitary factor 1 (PIT-1 or POU1F1), a protein factor produced in hypothalamic nuclei (*Carsai et al.*, 2012). In cattle, it was shown that a *Hinf*I polymorphism located in exon 6 of PIT1 gene may have significant influence on milk quantity. In particular A allele was associated with a higher milk yield and could be a valuable genetic marker for improving milk quantity in cattle (*Edriss et al.*, 2009; *De Mattos et al.*, 2004; *Selvaggi and Dario*, 2011). In contrast with our study results was association between Pit-1 gene polymorphism (HinfI) and milk production traits found in several studies of Holstein cattle. In study *Heidari et al.* (2012) was similarly for associations relationship evaluation used GLM procedure, while the authors found in Holstein cows population significant (P<0.05) effect of Pit-1 genotype on milk yield. Animals with AB genotype produced more milk than BB genotype. *Doosti et al.* (2011) reported also for Holstein cattle, that the Pit-1/HinfI genotype could be use in fertility and create the next generation for increase in milk production and growth of animal. In contrary in study of Brown Swiss population (*Ayetkinom and Boztepeom*, 2013) was not observed the effect of Pit-1 polymorphism on milk yield and composition (P>0.05).

CONCLUSIONS

Selection for animals with some of the Pit-1/HinfI gene genotype could result in advantages for production traits of dairy cattle. The Pit-1 gene as a regulator of growth hormone and prolactin genes expression is necessary for mammary gland development and also for milk production traits. Because the results of our study doesn't suggest a potential significant effect of Pit-1 gene polymorphism on milk production traits, these associations should be validated in cattle population including the larger number of animals or involvement of other dairy cattle breeds. The animals breeding selection assisted with genetic markers can increase the production traits or optimize reproduction performance in dairy cattle.

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Mitochondrial DNA as a tool for identification of genetic diversity among domestic animals

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ABSTRACT

Mitochondrial DNA (mtDNA) is genetic marker that is often used in population and evolutionary biology. The best preserved part of the D-loop is its central part while other segments are subject to change. Busha is autochthonous breed of cattle that is bred extensively on the territory of Republic Croatia. During the last century there has been significant reduction in her size which has led to an endangerment of the breed. Busha is the part of cultural heritage and valuable gene source, which is the one of the reasons for its preservations. Research was conducted on 15 samples of Busha. Samples were collected on five different locations on territory of Croatia. Research was carried out on the most variable part of mtDNA (D-loop). The results of the research revealed the variability in the mtDNA D-loop sequences. The most common substitutions within the D-loop were C/T and A/G substitutions. Degree of genetic divergence within population is nearly 27%. The aim of this paper was to examine efficiency of mtDNA as a molecular marker in the analysis of genetic diversity among animal population. In this research we have used population of busha.
(Keywords: Buša, genetic diversity, molecular marker, mitochondrial DNA (mtDNA))

INTRODUCTION

Marker systems are developing depending on the required type of DNA identification, reliability, specifics and analysis speed (*Ivanković,* 2005). Molecular markers are identified DNA sequences that can be found on the specific locations in the genome and are transferred according to standard inheritance laws, from one generation to the next (*Guimaraes et al.,* 2007). Mitochondrial DNA is an important marker in determining genetic diversity among animals. There are several reasons why mtDNA is used as a molecular marker; those are the absence of recombinations, simple organization, maternal inheritance and a high degree of mutation in relation to nuclear DNA (*Ballard and Rand,* 2005). It is an important marker for determination the genetic diversity among animals and also can be used for creating tree that follows movement of mothers' line and leads to the first mtDNA. *Galtier et al.* (2009) state that the paternal mtDNA is removed earlier, during and after fertilization. With this method it is ensured that the organism contains mtDNA from only one parent and it is possible to follow the line to the first female unit. Mitochondrial genes represent a string of genetic information all the way back to the first female unit or a group of female units (*Ballard and Rand,* 2005). It is a highly conserved and congealed circular molecule and located in the mitochondrial matrix. The size of an average molecule is 16.5kb, but it varies and is dependent on the species (*Kukat et al.,* 2011). In cattle it is 16 338 base pairs long, in horses 16 660 base

pairs, and in chickens 16 782 base pairs. Analysis of mtDNA contributes to the evidence of domestication places. The presence of nucleotide substitution in this region is 2.8 to 5.0 times larger than in other mtDNA regions (*Ivanković,* 2005; *Soares et al.,* 2013). The control region, the D-loop, represents the most variable part of mitochondrial DNA. It is one of the reasons for its use in genetic research. The D-loop is the main control region for mitochondrial DNA expression. MtDNA also gives information at the intercontinental level (*Lenstra et al.,* 2014). Determining the mtDNA sequence variations in the control region can be used as a very useful tool for clarification of the species and diversification of cattle breed.

Busha is an autochthonous Croatian breed of cattle. It is extensive breeding in the areas of Lika and Dalmatia (*Konjačić et al.,* 2004; *Simčić et al.,* 2008). Busha is not present exclusively in the area of Lika, it can also be found in the regions of Papuk, Psunj, Žumberak and the Krka National Park. *Međugorac et al*. (2008) state that there are several subpopulations of the Busha that inhabit areas of Croatia, Bosnia and Herzegovina, Montenegro, Serbia, Kosovo, Albania, Macedonia, Romania, Bulgaria, Greece, Turkey and countries of the Near East. Their morphology has been determined in several studies: *Adametz*, 1895; *Frangeš*, 1903; *Ogrizek*, 1930; *Ogrizek*, 1941; *Rako*, 1943; *Rako* 1947; *Šmalcelj and Rako*, 1955; *Šmalcelj*, 1956; *Puškaš*, 1983; *Šic et al*., 1994; *Konjačić et al*., 2004 (*Bulić et al.,* 2007). Busha is a breed with crude constitution and small physical frame. The color of the hairs is single color brown, red to black with a stripe on the back which is in contrast with the basic color. "Doe snout" is a characteristic of the breed, i.e. dark pigment in the mucous skin with a white hairy ring around it. Ridge height is 100 to 110 cm, body mass of cows is 250 kg, while the bulls can weigh up to 300 kg. Horns are short with a light coating around the base and black tips (*Čačić et al.,* 2012). The genetics of the Busha was determined, on the DNA sequence and blood protein polymorphism level, by *Ivanković et al*., 2004; *Konjačić et al*., 2005. Development of modern cattle production is based on neglecting the animal genetic limits. The consequence of that is full usage of useful genes to the degree that it represents a danger for the animal health. Autochthonous breeds has a high economic significance, as a gene banks they are indispensable tool in attempts to repair and improve genetic status of modern and high productive breeds of cattle.

MATERIAL AND METHODS

The research was conducted on 15 cattle of the Busha breed. The blood was collected by puncturing the jugular vein and placed in tubes with an EDTA coagulant. The blood samples were frozen within 3 hours after collection and kept at a temperature of 4 °C up to that point. Molecular and genetic analysis of the collected samples was conducted at the biological research lab of the Faculty of Agriculture in Osijek.

DNA isolation was conducted from 200 µl of homogenized blood using the phenol-chloroform extraction method (25:24:1) (*Ausubel et al.,* 2000). A TE buffer was used for blood plasma washing and leukocyte separation (Tris-EDTA, pH 8.3). Lysis buffer and proteinase K were used for destroying leukocyte and FOR protein removal. DNA washing was done twice using phenol-chloroform-isoamyl alcohol (PCI, 25:24:1) and chloroform-isoamyl alcohol (CI, 24:1). The isolated DNA was dissolved in 96% alcohol and then washed with 70% alcohol. The DNA acquired this way was dissolved in 25 µl of deionized filtered H_2O. Isolation check was done using electrophoresis on 2% agarose gel.

Figure 1.

Areas where were collected samples for analysis

The PCR conditions were 5 min of initial denaturation at 95 °C, followed by 35 cycles of elongation at 95°C within 50 sec, 61 °C within 50 sec and 72 °C within 50 sec, final extension at 72 °C during the 6 min. PCR reaction was prepared in 20 µl of mixture consisting the following: 12.1 µl ddH_2O, 2.0 µl buffer,1.2 µl $MgCl_2$, 1.0 µl dNTP, 1.0 µl F primers, 1.0 µl R primers, 0.2 µl Taq polymerase and 1.5 µl DNA. Two primer pairs were used to amplify the D-loop region MITb1 - (59-CTGCAGTCTCACCATCAACC-39) and MITb2 - (59-CTCCTCGGACAAGATATTAG-39).

PCR products were sent to South Korea for sequencing (Macrogen inc.). Computer program ClustaW was used for sequence alignment (*Thompson et al.,* 1994). Phylogenetic connection between units within a population was determined using a neighbor-joining (NJ) algorithm (*Tamura and Nei,* 1993). Bootstroop levels within the tree were determined with 1,000 repetitions. All the stated analyses were made using the computer program MEGA, version 3.1 (*Kumar et al.,* 2004). Analyses of molecular variance (AMOVA), FST values, as well as nucleotide differences (*Nei,* 1978) are done using the program Arlequin ver. 3.01 (*Excoffier et al.,* 2006).

RESULTS AND DISCUSSION

Conducted research has revealed the variability in the mtDNA D-loop sequences. Genetic structure within the studied population was examined using the AMOVA method. The method was made based on the allele content of different haplotypes, as well as their frequency. It is widely used tool for quantifying the various levels of population structure to patterns of genetic variation. First research which has included comparison of mtDNA different cattle breeds was conducted by *Bradley et al*. (1996). Research which was conducted till now showed that genetic variability declines with increasing distance from the domestication sites (*Lenstra et al.,* 2014). Cattle MtDNA D-loop sequences are divided into five mtDNA haplogroups: T, T1, T2, T3 and T4. T3 haplogroup is the most frequent group and it presents the dominant haplotype group.

Kantanen et al. (2009) has investigated geographical patterns of mtDNA diversity of Eurasian taurine cattle (*Bos taurus*).

Table 1.

Types of nucleotide substitution in the D-loop region of the investigated cattle

Nucleotide substitution	***Number***	***Percentage (%)***
C/T	211	55.2
A/G	148	38.7
A/C	10	2.6
G/T	2	0.5
C/G	7	1.8
A/T	4	1.0
Total	382	100.00

The *Table 1*. shows that the largest numbers of nucleotide substitutions in the D-loop region are C/T and A/G substitutions. There was 211 C/T substitution which represents 55.2%, and there are 148 A/G substitutions which are 38.7%. These two substitutions are the most common in D-loop. This substitution is also a characteristic of some other native breeds of cattle in the region (Cika), so it indicates a phylogenetic connection and the possibility of uncontrolled cross breeding in the very early stages of domestication. *Lenstra et al.* (2014) has shown that geographically differentiation of cattle mtDNA is stronger than in other domestic animals. *Cai et al*. (2014) conducted research which has included the European and Near Eastern domestic cattle and show the haplogroup distribution pattern of Chinese domestic cattle. Research which has conducted by *Ludwing et al*. (2013) included the investigations of six novel mitochondrial genes from the White Park Cattle. *Ivanković et al*. (2010) has conducted similar research. They investigate levels of genetic variability between Istrian cattle and Slavonian – Syrmian podolian cattle using microsatellites and D-loop as genetic markers. They have analyzed proximal part of the D-loop region. *Ivanković et al*. (2014) observed phylogenetic relationship of Croatian autochthonous cattle breeds. They have analyzed D-loop region of Busha, Istrian, and Slavonian - Syrmian podolian cattle populations. Results of their research have show that there is high level of mtDNA diversity in Busha population.

Table 2.

Genetic variation within the invastigation Busha population

	B14	**B15**	**B21**	**B18**
B14	-	***	***	***
B15	0.0293	-	n.s.	n.s.
B21	0.0265	0.0287	-	n.s.
B18	0.0264	0.0278	0.0259	-

***P <0.001

The samples were grouped regarding the collection area. There were 4 samples collected from North part of Croatia (B14), 6 samples from Dalmatia region (B15), 1 sample from

Istria region (B18) and 4 samples from Lika region (B21). Data were analysed using MEGA version 3.1., while FST values and analysis of molecular variance were made using Arlequin version 3.01. A statistically significant difference was detected within the population ($P<0.001$) concerning genetic variability between unit B14 and the other units in the population. Other units display a comparable level of genetic variability (average of 0.27%), which indicates approximate homogeneity and genetic stability of the population. The stated results point to a conclusion that unit B14 in not a Busha, it is instead a crossbreed which is similar to the Busha externally, but genetically it belongs to another breed, most probably a cross between several breeds. The reason for making that conclusion is the occurrence of statistically significant genetic difference which was determined between unit B14 and other individuals within the Busha population.

CONCLUSIONS

The largest numbers of nucleotide substitutions in the D-loop region are C/T and A/G substitutions. A statistically significant difference was detected within the population ($P<0.001$) concerning genetic variability between unit B14 and all the other units in the population. Other units display a comparable level of genetic variability (average of 0.27%), which indicates approximate homogeneity and genetic stability of the population. Conservation of genetic resources today is set as the imperative in livestock production. Busha represents a valuable genetic resource that must be protected and preserved. Information from mtDNA has great importance for conservation genetics in autochthonous cattle breeds.

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Direct and indirect genetic indices for milk coagulation properties in Italian Holstein Friesian sires

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ABSTRACT

Aim of this study was to define direct and indirect indices for milk coagulation properties (MCP) in Italian Holstein Friesian sires. A total of 315,700 individual milk samples from 49,183 cows were collected in 479 dairy farms from September 2011 to February 2014. Rennet coagulation time (RCT) and curd firmness (a_{30}) were predicted using mid-infrared spectroscopy. Sire breeding values (EBV) for RCT and a_{30} were estimated using a repeatability single-trait animal model, which included herd-test-day, days in milk, age at parities and season of parity as fixed effects, and cow permanent environment and animal as random effects. The direct genetic index for milk coagulation ability (IAC) was defined as the combination of EBV for RCT and a_{30} with equal weight, and then expressed on a scale with mean 100 and standard deviation of 5. A stepwise method was chosen to combine official EBVs for traits published by the Italian Holstein Friesian Cattle Breeders Association in a genetic index able to predict the direct IAC. Only sires with at least 10 daughters (scored for MCP) in 5 different herds were considered. The official EBVs retained by the regression analysis were those of protein and fat content, somatic cell score and the genetic variants for k-casein. Results of direct and indirect selection for MCP are presented.
(Keywords: genetic evaluation, milk coagulation properties, Holstein Friesian bulls)

INTRODUCTION

Despite cheese manufacture is the first destination of milk produced in Italy, the three major Italian dairy breeders associations (Holstein, Brown Swiss and Simmental) have not provided yet a direct genetic selection index to improve the milk aptitude to be transformed in cheese. Several studies have been published on cheese processing but they were performed only on a small number of animals, using bulk milk or with laborious procedures that can hardly be applied in routine (*Annibaldi et al.,* 1977*; Zannoni and Annibaldi,* 1981*; Aleandri et al.,* 1989; *Malacarne et al.*, 2006; *De Marchi et al.*, 2008). Recently, mid-infrared spectroscopy (MIRS) has been proposed as a cheap technology to predict milk coagulation properties (MCP) at population level (*De Marchi et al.*, 2009). The use of MIRS has made possible the storage of milk spectra and the development of prediction models for MCP (*Dal Zotto et al.*, 2008; *De Marchi et al.*, 2012; *De Marchi et al.*, 2014). Moreover, MCP have been found to have an exploitable additive genetic variation in dairy cattle populations, and estimated heritabilities from 15 to 41% (*Ikonen et al.*, 2004; *Cassandro et al.*, 2008; *Vallas et al.*, 2010). Therefore, the

improvement of MCP through genetic selection is feasible. Aim of this study was to define direct and indirect genetic indices for MCP in Italian Holstein Friesian sires.

MATERIAL AND METHODS

Phenotypic data

In the summer of 2011 the calibration curves for MCP were installed in Milko-scan FT6000 (*Foss Electric A/S*) of the Regional Breeders Association (Padova, Italy) for routine prediction of MCP as reported by *De Marchi et al.* (2012). Authors estimated satisfactory accuracies for the prediction equation of MCP, with coefficients of determination in cross-validation of 0.76 and 0.70 for RCT and a_{30}, respectively. Moreover, every 45 days a ring test is carried out by the 2 laboratories of the Regional Breeders Association to evaluate the effectiveness of MCP models and to reduce bias between FTMIR instruments and reference data (*De Marchi et al.,* 2012).

From September 2011 rennet coagulation time (RCT) and curd firmness (a_{30}) become available for cows reared in Veneto region and in April 2014 315,700 individual milk samples from 49,183 Holstein-Friesian cows collected in 479 dairy farms during monthly test-day milk recording were used for the estimation of breeding values for MCP.

Direct selection index

After discarding samples outside biological ranges and levels of fixed effects with low frequency, the Intermizoo SpA AI company (Padova, Italy) analyzed RCT and a_{30} with a repeatability single-trait animal model, which included herd-test-day, days in milk as classes of 15 days each, classes of age at parities and season of parity as fixed effects, and cow permanent environment and animal as random effects. Breeding values for sires with daughters with information on MCP were estimated using VCE6 software (*Groeneveld et al.,* 2008). Variances for RCT were set up to 2.780 (genetic), 4.410 (permanent environment) and 6.184 (residual), and those for a_{30} were 15.935 (genetic), 19.860 (permanent environment) and 30.170 (residual). The Intermizoo SpA AI company started in January 2012 to publish a direct selection index (IAC), which combines RCT and a_{30} with equal weight. The index is expressed on a scale with mean 100 and S.d. 5.

Indirect selection index

Currently, direct measures of MCP are available only for cows reared in Veneto region, and EBV are published only for bulls belonging to Intermizoo SpA AI company. The collection of MCP at national level is still under definition and evaluation, and the ANAFI has started to develop an indirect selection index to get round the lack of MCP data, and to point out the attention of milk-producers on those characteristics. A stepwise method was chosen to combine official EBVs of traits published by ANAFI in a selection index able to predict the direct IAC. Only bulls with at least 10 daughters (scored for MCP) in 5 different herds were retained for the regression. The official EBVs considered were those of protein and fat yield, protein and fat content, udder depth, somatic cell score (SCS) and the genetic variants for k-casein. Protein and fat contents, were considered as a combination as reported in the formula that predict cheese yield of Grana Padano cheese at 6 mo of ripening [CYgp = 2.833 + 0.711*genetic base of fat% + EBV fat%) + 0.179*(genetic base of protein% + EBV protein%); see *Aleandri et al.* (1989)].

RESULTS AND DISCUSSION

Descriptive statistics of phenotypic records used for the genetic evaluation of April 2014 is reported in *Table 1*. Mean for milk yield (29.7±9.5 kg/d), fat content (3.85±0.80%) and protein content (3.42±0.43%) of Holstein-Friesian cows reared and sampled in Veneto region are consistent with official national statistics (30.6 kg/d, 3.72% and 3.38%, respectively), reported by *ANAFI* (2014). Rennet coagulation time and a_{30} predicted by MIRS averaged 22.3±5.5 min and 23.0±10.6 mm, in agreement with values reported by *Tiezzi et al.* (2013) who used a sample of data to estimate (co)variance components.

Table 1

Descriptive statistics of milk coagulation properties and production traits of 49,183 cows reared in Veneto region and used to calculate the direct selection index

Trait	Mean	S.d.	CV (%)	Minimum	Maximum
RCT (min)	22.3	5.5	24.6	3.0	40.0
a_{30} (mm)	23.0	10.6	45.8	0.0	60.0
Milk (kg/d)	29.7	9.5	31.9	3.0	92.8
Fat (%)	3.85	0.80	20.82	1.50	9.00
Protein (%)	3.42	0.43	12.43	1.01	6.88
SCS	3.09	1.90	61.39	-3.64	9.64

Table 2

Pearson correlations of EBV for rennet coagulation time (RCT), curd firmness (a_{30}), direct index for milk coagulation ability (IAC) with official selection index (PFT) and EBVs of 683 Holstein bulls

Trait	RCT (min)	a_{30} (mm)	IAC
RCT (min)	-	-0.900	-0.977
a_{30} (mm)	-0.900	-	0.972
IAC	-0.977	0.972	-
Italian Selection index (PFT)	-0.103	0.153	0.130
Milk (kg/d)	0.036^{ns}	-0.152	-0.093
Fat (%)	-0.157	0.258	0.210
Protein (%)	-0.012^{ns}	0.285	0.145
Fat (kg/d)	-0.126	0.125	0.129
Protein (kg/d)	0.030^{ns}	0.041^{ns}	0.004^{ns}
SCS	-0.211	0.170	0.197
Udder composite (ICM)	-0.032	-0.002^{ns}	0.016^{ns}

^{ns}not significantly different from zero

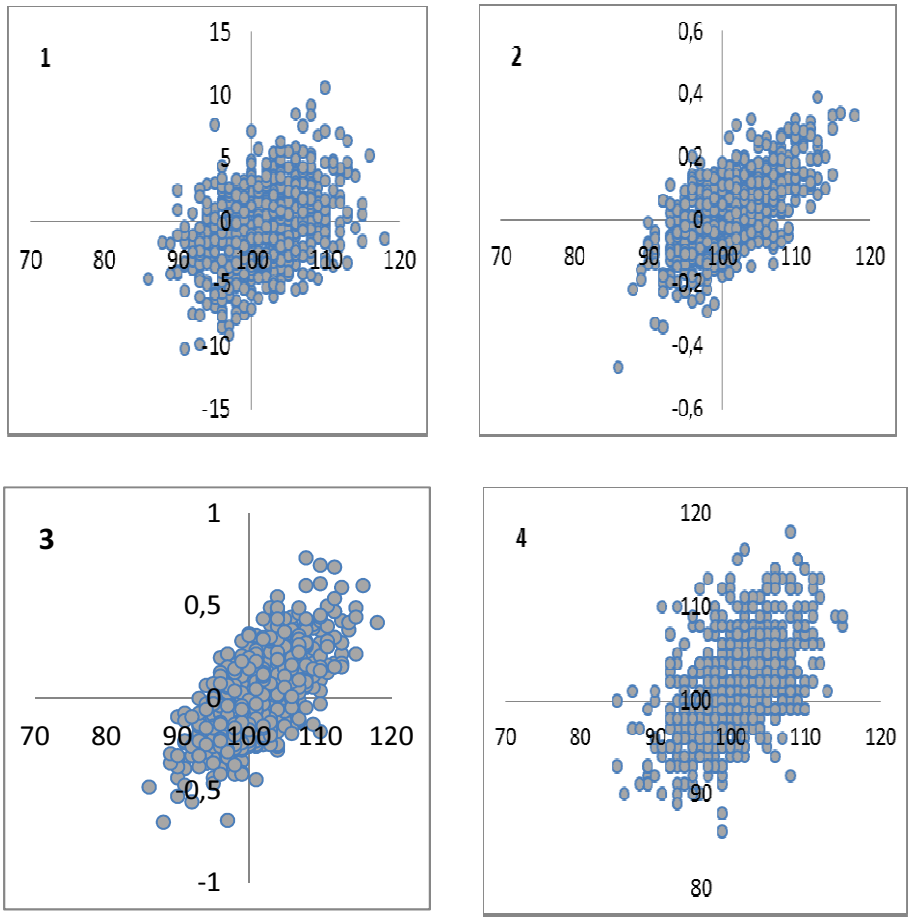
Direct selection index (IAC) showed low to moderate Pearson correlations with the official EBVs published by ANAFI. The highest correlations were with fat content (0.210) and SCS (0.197), and the correlation with the national selection index (PFT) was 0.130 and favourable (*Table 2*). Rennet coagulation time and a_{30} were correlated with the

same strength of the direct index to the EBVs of milk production traits, except for EBVs of milk yield; in this latter case, the relationship between RCT and milk yield was around zero, whereas those of a_{30} and IAC with milk yield were unfavourable and equal to -0.152 and -0.093, respectively.

The final model of the stepwise regression included, as best predictor of IAC, the EBVs of protein and fat content combined in the CYgr, the EBVs of SCS, and a penalty for bulls with "AA" and "AB" genetic variants of k-casein. The rank correlations between the indirect index (ITC) and EBVs for MCP were moderate and equal to -0.23 (RCT), -0.38 (a_{30}) and 0.31 (IAC), thus re-ranking of bulls are expected. The ITC had high correlations with traits used in the regression and equal to 0.58 (protein content), 0.63 (fat content) and 0.54 (SCS; *Figure 1*).

Figure 1

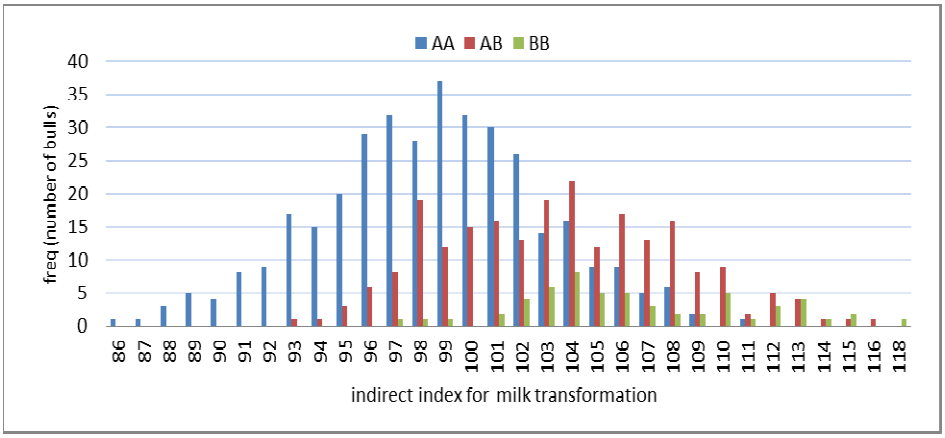
Dispersion plot between EBVs of a_{30} (1), protein content (2), fat content (3) and SCS (4) with the indirect selection idex (ITC) estimated by ANAFI



Bulls with desirable variant of k-casein showed higher ITC, with means of 98.6, 103.5 and 106.6, respectively, for bulls with AA, AB and BB k-casein variants (*Figure 2*).

Figure 2

Distribution of indirect index for milk transformation (ITC) for the genetic variant of k-casein



CONCLUSIONS

Implementation of MIRS models for MCP in the laboratory of Veneto region allows the collection of routine data that can be used as phenotypic records for genetic evaluations due to their genetic variance and importance in the Italian dairy systems. To now, an extensive sampling of traits related to cheese yield was limited because expensive and time consuming, but MIRS technologies provided, also for those traits, an effective and low-cost source of information. The Veneto region experience showed that direct genetic evaluation of MCP is feasible. To start a national genetic evaluation for MCP some efforts are needed: first, other regional laboratories have to install prediction equations of MCP, and then ring test and validation between laboratories have to be routinely performed. When problems related to data collection and data flow will be solved, the bodies responsible for the calculation of official EBVs have to develop the optimal selection index to improve the aptitude of milk to be transformed in cheese.

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Preferences of Istrian sheep udder shape type on farms that apply machine milking

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ABSTRACT

Istrian sheep is an indigenous endangered breed reared at extensive or semi-extensive farms in Croatia. The number of farms applying machine milking is increasing due to high quality dairy products. The objective of this work was to evaluate morphometry of the udder and milk flow kinetics in the Istrian sheep in Croatia, and to explore possible preferences of udder shape type on farms that apply machine milking. Using Lactocorder© (WMB; Switzerland) we measured milk flow kinetics in five commercial herds. Using digital photographs of the posterior view of the udder and Image Tool software we measured udder height, width, cisternal part below the teat orifice and the angle that teat closes with the vertical axis of the udder in eleven herds. Breeding values were estimated using univariate animal models and REML (Restricted Maximum Likelihood). Istrian sheep breed in Croatia has excellent udder shape for machine milking: desirable angle that teat closes with the vertical axis of the udder, and cisternal height below the teat orifice is small. Ewes that are machine milked have higher udder, lower cisternal part below the teat orifice, and teats are more vertically implanted, which is the udder conformation beneficial for more efficient machine milking. BLUP value differences indicated that machine milked herds tend to have ewes with smaller cisternal part below the teat orifice that are of less udder height in the beginning of lactation and wider at the end of lactation, although there is no official selection of udder shape.
(Keywords: udder morphometry; milkability; BLUP; dairy ewe)

INTRODUCTION

Istrian sheep (IST) is an autochthonous endangered breed, according to FAO, EU and IUCN categorisation. Registered population of 2 515 animals on 38 farms in Croatia makes it the smallest autochthonous sheep population used in dairy production in Croatia (*Mulc et al.,* 2012). Most of ewe milk is processed into hard artisanal cheese and crude on small family cheese dairies. The production is extensive or semi-extensive, with average herd size of 55 animals (*Mulc et al.,* 2012), and only few counting more than 200 animals. Herd size limitation exists due to milking effort, since the machine milking in IST is present only to some extent. In the last decades the number of sheep farms with machine milking is increasing, therefore it is important to know if IST is suitable for machine milking. Benefits of machine milking of ewes are maximal milk yield of better hygienic properties than properties of hand-milked milk, and easier stripping (*Dzidic,* 2013). Effective milkability depends on udder morphology (*Labussière,* 1988) and is important for sustainable milk production because it affects functional life span of the

animals (*Casu et al.,* 2006). Milkability can be evaluated by analysis of the milk flow curves and milk flow parameters that describe the physiological response of ewe to machine milking (*Mayer et al.,* 1989; *Bruckmaier et al.,* 1997), and by analysis of udder morphometry (*Labussière,* 1988; *Fernandez et al.*, 1995). The need of vertically implanted teats at the lowest point of the cistern as improved udder traits (*Labussière,* 1988) is recognized in the selection objectives of ovine breeding schemes (*Casu et al.*, 2006; *Marie-Etancelin et al.*, 2006). The reason for the increased interest was "baggy udder", found in sheep selected for high milk yield. Milking of these "baggy udders" is not efficient because part of the cisternal milk remains below the teat orifice unless the milker applies manual manipulation of the udder during stripping (*Bruckmaier et al.*, 1997). Additionally, horizontally implanted teats cannot hold the weight of the milking unit, and it tends to fall off. That kind of additional manipulation during milking prolongs the total milking time of the herd, with milking already being one of the most time-demanding procedures on ewe milk farms. Therefore, the mammary gland morphology is an important factor in determining the aptitude for the machine milking of ewes.

In order to evaluate suitability of the Istrian sheep for machine milking, our objectives were to evaluate morphometry of the udder from digital photographs of the posterior view of the udder, to evaluate milk flow kinetics in the Istrian sheep on the farms that apply machine milking, and to explore possible preferences of udder shape type on farms.

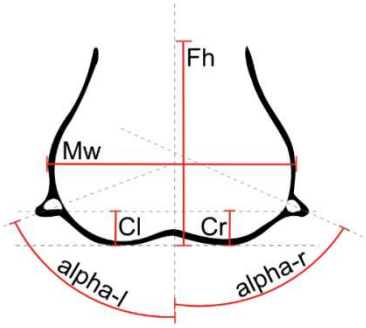
MATERIAL AND METHODS

Milk flow kinetics during machine milking of IST was measured in five commercial herds using Lactocorder© (WMB; Switzerland) specially calibrated for milking of the ewes (*Dzidic et al.,* 2004). The milking time, milk yield, peak, and average flow rate were obtained in early (first 3 months), mid- (months 4 and 5) and late lactation (months 6 to 8) during year 2010. The animals were milked twice a day and there were 148 morning and 418 evening measurements ranging from eight to 188 days in lactation. Milk production lasted 8 h during the day and 16 h through the night. Milking units were used at a milking vacuum of 37 kPa, pulsation rate 120 cycles/min and pulsation ratio 50:50. The milk was collected in buckets. Teat cups were attached to the udder without previous touching of the udder. Milking routine was finalized with manual udder massage and lifting of the lowest part of the udder in order to position the teats as low as possible when the milk flow dropped below 100 g/min with teat cups still attached.

Digital photographs of 258 ewe's posterior view of the udders were taken prior to evening milking on eleven commercial farms in Istria three times during lactation. Six of the farms performed milking by hand and five farms used machine milking. External udder shape was measured from the digital photographs using Image Tool software as shown in *Dzidic et al.* (2009): udder height (Fh); udder width (Mw); part of the left (Cl) and right (Cr) udder cistern that is below the teat orifice; and the left (Alpha-l) and right (Alpha-r) teat angle, as the angle declines from the vertical axis of the udder (inter-mammary groove) (*Figure 1*).

Figure 1

Udder shape measurements



Udder height (Fh); udder width (Mw); part of the left (Cl) and right (Cr) udder cistern that is below the teat orifice; and the left (alpha-l) and right (alpha-r).

Descriptive statistics for data and development of the fixed part of the model were obtained using GLM procedure (*SAS,* 2011). Breeding values were estimated using univariate animal models and REML in AS-Reml program release 3 (*Gilmour et al.*, 2009). Fixed environmental factors to be included in the models were additionally explored in AS-Reml program release 3, according to results of building successively univariate analysis of variance. Udder shape traits (Fh, Mw, Alpha, Cis) and milk flow kinetics (Mt, My, Avgm, Mmf) during machine milking were explored using the general univariate mixed model shown in Equation 1. Farm, litter size, number of lactation and day of measurement are defined as fixed influences in udder shape models. Milk flow kinetics model included additional fixed effect of milking interval. Modelling Cis and Alpha included additional fixed effect of the udder half with two levels. Additive genetic value of the individual and permanent environmental effect within the day of measuring were the random effects in udder shape models. Additive genetic value of the individual was the random effect in milk flow kinetics model. Additionally, Mmf model included random effect of permanent environment. Pedigree of IST is recorded by Croatian Agricultural Agency. In the genetic models, all available relationships of 22 042 identities for the period 1989 – 2012, spanning over 9 generations, were used.

Equation 1

$$y_{ijkln} = \mu + D_i + S_j + L_k + F_l + F_l{*}L_k + a_{ni} + p_{ni}{*}D_i + e_{ijkln}$$

Where: y_{ijkln} = individual observation of Fh, Mw, Alpha, Cis, Mt, My, Avgm, Mmf; μ = intercept; D_i = fixed effect of measuring day (i = 1, 2 and 3); S_j = fixed effect of litter size (j = 1 and 2+); L_k = fixed effect of the lactation number (k = 1, 2, 3, 4 and 5+); F_l = fixed effect of the farm (l = 1 to 11 for udder shape traits, and 1 to 5 for milk flow kinetics); a_n = the random additive genetic effect of animal; p_{ni} = the random permanent environmental effect within day of measurement (for Alpha and Cis); e_{ijkln} = the residual.

RESULTS AND DISCUSSION

Table 1

Mean values of morphometric and milk flow kinetics traits studied in Istrian sheep

	Mw	Fh	Alpha	Cis	Avgm	Mmf	My	Mt
Mean	13.5	14.1	29.4	1.4	0.5	0.5	0.5	1.2
SE	0.45	1.84	3.88	0.24	0.05	0.07	0.06	0.14

Mw - Maximum udder width (cm); Fh - Full udder height (Fh); Alpha - angle that teat closes with the vertical axis of the udder (^{o}); Cis - Height of the cisternal part below the teat orifice (cm).

Milk flow kinetics trait means are shown in Table 1. Average milk flow in Istrian sheep is appropriate, comparable to European dairy sheep, and supported by the conclusions on excellent udder shape. Mean average milk flow was similar as reported by *Casu et al.* (2008), and to the values reported for Lacaune and East Friesian (*Bruckmaier et al.*, 1997), or Istrian crossbreeds (*Dzidic et al.*, 2009), but lower than in Sardinian ewes (*Carta et al.*, 2000). Mean peak flow rate was lower than all the reported values: remarkably lower than in *Casu et al.*, 2008 (19.7 ml/s), lower than in French dairy ewes (*Marie-Etancelin et al.*, 2006), lower than that found for Slovak dairy ewes (*Tančin et al.*, 2011; *Kulinova et al.*, 2010; *Mačuhova et al.*, 2011) and in Istrian crossbreeds (*Dzidic et al.*, 2004; 2009). Peak flow rate mean was most similar to the Mmf of 75% Istrian crossbreeds that had the lowest Mmf in comparison with crossbreeds with lower percentage of Istrian genetic background as reported by *Dzidic et al.* (2004). Peak flow rate in Istrian sheep is lower than in European dairy breeds. Intrinsic factors influencing the peak flow rate, such as teat sphincter opening characteristics, can be improved through selection. However, environmental sources constant through lactation affecting the peak flow rate could be symptomatic of insufficient adaptation of milking setting or machine characteristics to the breed (type and shape of liners, diameters of milk lines and tubes, air entry flow), especially as the lactation stage advances and milk production declines. Mean milking time (Mt) was lower than the range reported for Lacaune and East Friesian (*Bruckmaier et al.*, 1997), and higher than reported for Slovak dairy ewes (*Tančin et al.,* 2011, *Kulinova et al.*, 2010; *Mačuhova et al.*, 2011). Mean milk quantity per milking was lower than the range reported for Lacaune and East Friesian (*Bruckmaier et al.*, 1997), and by *Casu et al.* (2008), or in Istrian crossbreeds (*Dzidic et al.,* 2004; 2009) but similar to total milking yield found for Slovak dairy ewes (0.32-0.55 kg: *Tančin et al.*, 2011; 0.41: *Kulinova et al.*, 2010; *Mačuhova et al.*, 2011) as could be expected.

The mean values of udder shape traits are shown n Table 1. The Fh mean increased in mid-lactation and decreased at the lactation end. The udder had the highest Fh in third lactation ewes. Maximum udder width and Cis means were decreasing towards the end of lactation. Alpha did not change within or among lactations, indicating the permanence of the teat angle measurement during the life time of the IST ewe, as well as independence on the udder milk content during the IST lactation. This means that only one measurement during life time would be enough in case of udder assessment for obtaining animals' breading values if such assessment was introduced to IST breeding plan. Cis mean was the lowest in the first lactation and was increasing for every following lactation, and it was highest for ewes in the 5th and later lactations.

Table 2

Mean differences of ewe mean measurements, and BLUPs of udder shape traits regarding type of milking applied on farm of Istrian sheep

	Machine milking			Hand milking		
	Mean ± SE	Min.	Max.	Mean ± SE	Min.	Max.
Mw	10.71 ± 0.12	7.56	15.84	11.27 ± 0.21	8.30	14.15
Fh	13.65 ± 0.14	8.83	21.41	13.02 ± 0.29	9.21	17.21
Alpha	38.17^{a} ± 0.77	7.31	74.29	42.62^{b} ± 1.17	13.00	82.01
Cis	1.33^{c} ± 0.04	0	4.16	1.76^{d} ± 0.07	0	4.40
B-Fh1	-0.11^{c} ± 0.024	-1.15	1.17	0.26^{d} ± 0.034	-0.46	1.60
B-Fh2	-0.10^{a} ± 0.022	-1.54	1.05	-0.21^{b} ± 0.031	-1.43	0.60
B-Fh3	0.03^{c} ± 0.021	-0.82	1.10	-0.07^{d} ± 0.014	-0.57	0.44
B-Mw1	-0.01^{c} ± 0.020	-0.74	0.94	0.14^{d} ± 0.022	-0.80	0.97
B-Mw2	-0.03^{c} ± 0.018	-0.60	0.79	-0.12^{d} ± 0.019	-0.75	0.39
B-Mw3	0.03^{a} ± 0.013	-0.56	0.80	-0.02^{b} ± 0.011	-0.40	0.53
B-Alpha	1.06 ± 0.444	-19.16	24.47	1.46 ± 0.402	-15.27	16.88
B-Cis	-0.02^{a} ± 0.030	-1.18	2.04	0.12^{b} ± 0.034	-1.17	2.00

Means in the rows with superscript differ regarding the type of milking applied: ^{a,b} $P < 0.001$; ^{c}, ^{d} $P < 0.01$. SE – standard error; Mw - Udder width (cm); Fh - Udder height (Fh); Alpha - angle that teat closes with the vertical axis of the udder (^{o}); Cis - Height of the cisternal part below the teat orifice (cm); B-Fh1- Udder height BLUP during the 1st measuring day (cm); B-Fh2- Udder height BLUP during the 2nd measuring day (cm); B-Fh3- Udder height BLUP during the 3rd measuring day (cm); B-Mw1- Udder width BLUP during the 1st measuring day (cm); B-Mw2- Udder width BLUP during the 2nd measuring day (cm); B-Mw3 - Udder width BLUP during the 3rd measuring day (cm) ; B-Alpha – BLUP value of the teat angle (^{o}) ; B-Cis – BLUP value of the height of the cisternal part below the teat orifice (cm).

When examining the means of udder shape traits measurements and BLUPs, we found differences between udder shape of ewes from farms that milk by hand and the farms that apply machine milking (Table 2). Significant differences of means between ewes milked by machine and by hand were found in teat angle and cistern height averages, but not in udder height and width averages across lactation. Teat angle averages across lactation, and range, were smaller in ewes on farms that apply machine milking. Cistern height was smaller in machine milked ewes as well, however, the range did not differ remarkably. All BLUP values showed differences, except for teat angle indicating that the farmers prefer similar teat position in both milking systems. The BLUP values for Fh and Mw were predicted separately for beginning, mid-, and late lactation. BLUP values for Cis were negative (-0.02) for machine milked ewes, and positive in hand milked ewes (0.12), showing the same pattern as the measurements:

smaller cisternal part below the teat orifice in machine milked ewes. BLUP values for full udder height in the beginning of lactation were negative in machine milked ewes (-0.11), opposed to hand milked ewes (0.26), indicating that sheep with shorter, compact udder are preferred at machine-milking farms. This result could also be related to the cistern size results, since the Fh measurement includes Cis as shown on Figure 1. Mid- and late lactation Fh BLUP values showed the opposite pattern, and the udder was higher in machine milked ewes (-0.10 and 0.03 respectively). Udder width BLUPs across whole lactation were showed wider udder tendency in machine milked ewes.

CONCLUSION

Istrian sheep breed in Croatia has excellent udder shape for machine milking: desirable angle that teat closes with the vertical axis of the udder, and cisternal height below the teat orifice is small.

Although there is no official selection of udder traits in IST, differences between udder shape of ewes from farms that milk by hand and the farms that apply machine milking were found, indicating that there are different preferences of the owners. Herds that are machine milked have ewes with higher udder, teats that are more vertically implanted, and lower cisternal part below the teat orifice, which is the udder conformation that is beneficial for more efficient machine milking. BLUP value differences indicated that machine milked herds tend to have ewes with smaller cisternal part below the teat orifice that are of less udder height in the beginning of lactation and wider at the end of lactation, which indicates possible selection of ewes that are milked more efficiently and easier on farms that apply machine milking.

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Preliminary results of chemical analysis of sow colostrum from first to ninth parity

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ABSTRACT

Chemical composition and quality of colostrum have a great influence on pigs' lifetime productivity and pre-weaning mortality. There are a different methods for determinate quality of colostrum, but some of them are not suitable for use at the farms. Method for assessing the quality of colostrum should be quick, simple and accurate. In today's intensive keeping condition pre-weaning period is the most sensitive and the highest losses of piglets are in that period of production. Quality of colostrum is the factor which has an influence on the decrease of that mortality. Research was conducted on 90 samples of colostrum collected from 33 PIC sows. Samples was collected regarding the parity (first, third and ninth) and time of the year (spring, summer and winter). The objective of this study was to examine the changes in fat, protein, lactose, dry matter (DM) and non-fat dry matter (NDF) amount in sows' milk regarding the number of parity and season as well as determining the quality of colostrum using the refractometry method.
(Keywords: sows, colostrum, chemical composition, piglets)

INTRODUCTION

The most critical period for surviving the piglets is within few hours after the farrowing. There are three different energy sources for newborn piglets, and they are glycogen, colostrum and transient milk. Fat and lactose from colostrum ensures enough energy for piglets until transient milk become available.

Colostrum is the first product of mammalian gland and it represents a key to survival of newborn piglets. It is rich with digestible nutrients since it contains functional proteins, immunoglobulins (Ig), fats, minerals and vitamins in order to ensure good health of piglets. It is secreted from the udder after farrowing. Chemical composition of colostrum is very variable and it change rapidly to the milk (*Rooke & Bland*, 2002). Colostrum is essential for survival of the piglets during the lactation period and also post weaning period (*King'ori,* 2012, *Rolinec et al.,* 2011; *Quesnel,* 2011; *Cabrera et al.,* 2010). *Devillers et al.* (2007) have shown the importance of colostrum during the pre-weaning period. They limited the amount of colostrum which piglets consume and which led to the increase of piglets' mortality to 82%. Colostrum contains immunoglobulins, minerals and growth factors. Immunoglobulins are important because they neutralize toxins, viruses and bacteria. They represent passive immune protection because they renew and strengthen immune functions (*Gálik et al.,* 2011). The most important substances of colostrum are immunoglobulins since they provide passive immunity to piglets. Until now, a lot of researches regarding the concentration of immunoglobulines

content in dependence of number of parity (*Inoue et al.,* 1980; *Tuchscherer et al.*, 2006; *Krakowski et al.,* 2002) were conducted.

If piglets do not take colostrum within few hours after farrowing, the interstinalne becomes impermeable to immunoglobulins. Piglets do not have fully developed immune system so they are sensitive to different kind of pathogens. If piglets do not get enough amount of colostrum they are going to be more sensitive to pathogens and hypothermia. Colostrum also provides gastrointestinal development, muscle protein synthesis and it is the strongest natural immune stimulator (*Božanić,* 2004). For the keeping of positive energy balance is important that piglets consume colostrum within 24 hours after farrowing. Chemical composition and amount of produced colostrum varies among the sows. Factors responsible for that variability can be divided into two groups: genetic and non-genetic (*Trakovická et al.,* 2005). Factors affecting the colostrum composition and yield are genotype, number of parity, nutrition, endocrine status and environment factors (*Farmer and Quesnel,* 2008). Nutrition is the factor which has the greatest influence on composition. The main role of colostrum is to supply piglets with immunoglobulines (*Godden,* 2008). Other components of colostrum are relaxin, IGF-2, IGF-1, leptin, prolactin and insulin (*Blum and Hammon,* 2000*; Bartol et al.,* 2008). Colostrum is also important for development of intestinal system, thermoregulation of piglets and it is important for immune transfer from the sow (*Xu et al.,* 2000; *Le Dividich et al.,* 2005). Research conducted by *Bartol et al*. (2008) showed that piglets fed enough amounts of colostrum had better reproductive performance later in life. High quality of colostrum is an important factor that affects the health of newborn piglets. *Rolinec et al.* (2011) investigated the change in chemical composition of colostrum within 12 hours after the farrowing. They have conducted research on 20 sows with different number of lactation and litter size. The highest content of dry matter was 2 hours after farrowing (21.91%) and its concentration was reduced within 12 hours on 18.74%. Concentration of crude protein was 13.59% and 12 hours after farrowing it was 8.85%. The lowest concentration of fat was 2 hours after farrowing (3.43%), and the highest 8 hours after farrowing (5.21%). The highest concentration of lactose was 10 hours after farrowing and it was 3.55%. The decreasing of protein content and dry matter and increasing the fat indicate the transition from colostrum to the milk. *Salobir and Rezar* (2009) said that there are 3.4% of lactose, 5.9% fat and 15.1% proteins in colostrum.

The most accurate method for evaluating the content of IgG in colostrum is radial immunodiffusion (RID). Visual assessment is not precise indicator of the antibody level because fat and proteins can change visual appearance of colostrum and they do not reflect the level of antibodies. Assessing the quality of colostrum by using a hygrometer is simple for use and economically acceptable. It measures the specific weight of colostrum using floating glass. A new method for measuring the quality of colostrum is the use of refractometer. Refractometry is an optical phenomenon that is based on the diffraction of rays of light on the border of two different environments in which the light spreads at different rates (*Chigerwe et al.,* 2008). Dry matter determined in that way represents water-soluble substance and it is measured with special instrument, refractometer. The advantage of this method is in simplicity, time of measure and small amounts of samples. Preliminary research indicates that the result of the 22% Brix or more indicates good quality colostrum *(Quigley et al.,* 2013; *Bielmann et al.,* 2010). Although Brix refractometer measures the amount of sugar in the sample, the result can be converted to the estimate of the total solids content of milk (*Dairy News,* 2010). Following is the equation developed by Penn State:

$$\text{The total dry matter} = (0.9984 \times \% \text{ Brix score}) + 2.077$$

MATERIAL AND METHODS

Collecting the samples

86 samples of colostrum from 33 sows (PIC hybrids) were collected. The samples were divided into three groups depending on the number of parity and time of the sampling. There were collected 40 samples from sows between first and third parity, 22 samples from sows between fourth and sixth parity and 24 samples from sows between seventh and ninth parity. Samples were collected during three time periods: spring, summer and winter. The first sampling was carried out during April 2013th year, the second one during the August 2013th year and the last one in the February 2014th. Samples were collected from the same gilts and sows in the group during the sampling period. Samples from the first group belong to the sows from first to the third litter (1^{st}), samples from second group belongs to the sows from fourth to sixth litter (2^{nd}), and last group of samples belongs to the sows from seventh to ninth litter (3^{rd}). All animals involved in this study were kept under the same feeding and keeping conditions. From each sow there was taken 10 ml of colostrum. Colostrum was taken from only one breast.

Preparing the samples

Sample was taken to the sterile tube and after the collection was stored at the temperature of -20 °C. Frozen samples for the refractrometry analysis were melted at the room temperature and blended. In the same way samples were prepared for chemical analysis.

Analysis with digital BRIX refractometer

For analysis used ATAGO PR-100 (measuring range 0–32% BRIX) device was used. After instrument calibration with distilled or deionized water the colostrum samples were measured. The duration of measurements was 1,5sec. Instrument measure the refractive index of the sample and instantly converts it to % BRIX units. Minimal amount of sample necessary for measurement is 100 µl (or cover prism in its entirety).

Chemical analysis of samples

Chemical analysis of collected samples was carried out on the MILKOSCAN FT120 (FossElectric). In this way the concentration of fat, proteins, lactose, non fat dry matter (NDM) and dry matter (DM) was determined.

RESULTS AND DISCUSSION

Table 1 show the results from analysis of colostrum samples taken from gilts and sows from first to ninth parity. The data were submitted to ANOVA and differences between groups were analyzed with Fisher's test. The results of present study do not correspond to the research of Mahan (1998) who suggested that concentrations of fat decrease with parity. Number of litter and sampling period had a significant effect on sow fat content in colostrum, but there was not found any statistically significant effect between these two factors. Chemical analysis indicated that levels of fat were the highest in the colostrum of sows from 3^{st} group. Moreover, there was found a statistically significant effect of sampling period on nonfat dry matter. The lowest average value was during the February while the highest was recorded during the August, with the exception of sows from 3^{rd} group. There was not found any statistically significant effect of sampling period and number of litter at the level of protein, lactose and dry matter. *De villers et al.*

(2007) showed that parity had influence on the milk yield; during the second and third parity yield was higher in comparison to first or later parity.

Table 1

Chemical composition of sows colostrum regarding the number of parity and time of sampling

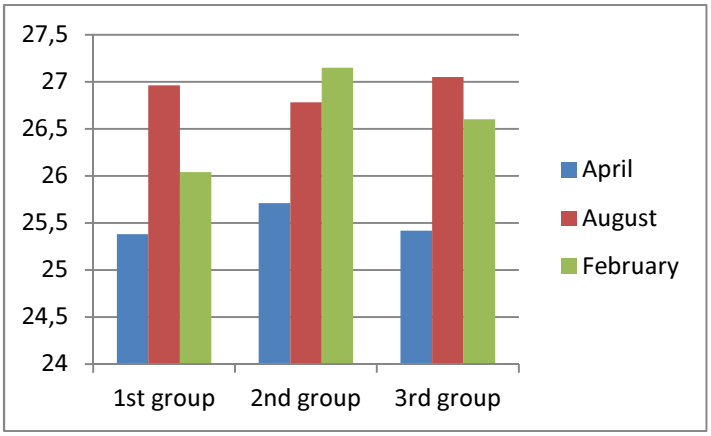
Number of parity	Time of sampling	n	Fat (%)	Proteins (%)	Lactose (%)	Nonfat dry matter (%)	Dry matter (%)
1.-3.	April	15	5,43±0,83^{ABC}	16.41±1.79	2.52±0.27	18.70±1.42^{BC}	52.75±6.02
	August	12	4,47±1,03^{D}	15.88±1.27	2.63±0.23	20.13±1.17^{A}	54.28±4.31
	February	13	5,32±0,82^{BC}	15.59±1.39	2.49±0.29	19.63±1.25^{AB}	52.94±4.57
4.-6.	April	8	5,34±0,71^{ABC}	16.04±2.09	2.61±0.38	18.46±1.62^{BC}	51.20±6.42
	August	8	4,85±0,48^{CD}	15.95±1.27	2.59±0.25	20.11±1.28^{A}	54.43±4.41
	February	6	5,66±0,55^{ABC}	15.09±1.23	2.45±0.31	19.02±1.14$^{AB C}$	50.80±4.84
7.-9.	April	10	6,05±0,81^{A}	15.55±1.34	2.65±0.21	17.98±1.17^{C}	49.99±4.95
	August	8	6,00±0,81^{AB}	15.36±2.52	2.67±0.51	19.51±2.37^{AB}	52.26±9.22
	February	6	5,76±0,97^{AB}	15.99±1.59	2.56±0.39	20.08±1.37^{A}	54.57±5.39
	P value number of parity		<0,001	0,705	0,605	0,651	0,666
	P value time of sampling		<0,05	0,614	0,316	<0,001	0,287
	P value interaction		0,234	0,705	0,964	0,551	0,638

A,B,C,D: different capital letters in the same column differ by Fisher's test (P>0.001)

BRIX value measurement of sow colostrum samples from the 1st group was in the rage of 25.38% during the April to the 26.96% during the August. Average values of 1st group were 25.38% (April), 26.96% (August) and 26.04% (February). Average values of colostrum samples from 2nd group were 25.71% (April), 26.78% (August) and 27.15% (February). Average values were 25.42% (April), 27.05% (August) and 26.6 (February). Our results correspond to the results of *Deelen et al*., 2014, *Quigley et al*., 2013, *Bielmann et al*. 2010.

Figure 1

Change of BRIX value regarding the sows' parity



CONCLUSIONS

Colostrum is a factor which affects the health of piglets and determines a complete production cycle and therefore the determination of its quality is of great importance. The paper presents the preliminary results of chemical composition of sows' colostrum, regarding the sows parity and seasonality, measured with standard chemical tests and BRIX refractormeter. Considering that the amount of dry matter corresponds to the concentration of imunoglobuline G we would like to compare the BRIX refractrometer method with laboratory measuring of chemical composition. Measuring the chemical composition of colostrum using this method could help the producer to replace bad quality colostrum and minimize the losses during production cycle.

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Comparative analysis of meat and fat tissue of Mangalica and meat-type hybrid pigs by means of Computerised Tomography

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ABSTRACT

The tissue composition of 6-6 Mangalica and meat-type pigs was determined by serial in vivo CT scanning. X-ray density frequency curves were developed from the whole body to determine the different tissue volumes. The calculated meat to fat ratio values were 3.4, 3.2 and 1.9 (meat-type pigs), while those of fat-type pigs were 1.0, 0.6 and 0.5, respectively, in the average weight of 30, 60 and 90 kg. In these weights, the meat percentage values were 35.5, 30.9 and 28.5 of the Mangalicas and 61.5, 57.7 and 53.2 % of meat-type pigs. The investigation of the tissue development in the body was carried out by means of 3D histograms. In the cross-sectional images the surface of the m. longissimus dorsi and also the m. semitendinosus was determined and the fat thickness at the back and the rump were measured. The intramuscular fat content was characterised by the average X-ray density value.
(Keywords: Pig, meat-type, Mangalica, tissue composition, computerized tomography)

INTRODUCTION

Pigs can be regarded as a highly variable species with as many as 500 breeds known worldwide. Its biological diversity is demonstrated by the differences found in the tissue and body composition of the meat- and fat-type pigs. Carcass traits have been extensively studied concerning meat-type pigs, however Mangalicas are much less known from this aspect. The growing intensity of the two genotypes is considerably different and among other factors it is detectable through the feed conversion ratio and tissue composition. The fattening period of the Mangalica is double than that of the meat-type pigs until reaching the same final weight.

Based on test slaughters of different genotypes comparative studies were already accomplished (*Adilovic et al*., 1985; *Rede et al*., 1986; *Rühl*, 1971). The specific characteristics of Mangalica meat and fat were also investigated (*Ender et al*., 2002). Having the same carcass weight Mangalica pigs attain substantially less skeletal muscle and more fat. They also show significantly higher fat thickness and lower loin depth compared to the meat-type pigs. According to *Straadt et al*. (2013), when crossing Duroc and the crossbreed Landrace/Yorkshire with the alternative breeds Iberian and Mangalica the pork loins of the offspring did not differ generally in odour, appearance or flavour/taste when compared with the traditional DLY crossbreed (*Straadt et al*., 2013).

Computed tomography scanning has the ability to describe and follow the changes in the whole body composition across time in live animals, in a non-invasive and non-

destructive manner (*Bunger et al*., 2011). Having the advantage of an *in vivo* application, multiple measurements can be taken on the same individuals. Tissue volumes can then be transformed into very accurate tissue weights by multiplying them by the tissue density values (*Lambe et al*., 2013). This procedure was applied by numerous investigators for body composition determination and for making selection decisions in pig research (*Horn et al*., 1997; *Szabó et al*., 1999; *Thompson and Kinghorn*, 1992; *Vangen*, 1992).

Parallel with the current study, cine MRI examinations were performed to characterise the heart performance of the two pig genotypes (*Petrási et al*., 2003). Acceording to the authors Mangalicas possess considerably higher circulatory reserves than the meat-type pigs.

The objective of the present *in vivo* analysis by means of computerised tomography was to conduct a comparative study of tissue composition of meat-type and Mangalica pigs weighing 30, 60 and 90 kg, respectively.

MATERIALS AND METHODS

During the course of our investigation six castrated meat-type (Hungarian Large White × Belgian Landrace × Pietrain × Norwegian Landrace) and six castrated 'dirty' white Mangalica pigs were measured at different weight categories (30, 60 and 90 kg). Meat-type pigs were set into small groups of 25–30 individuals applying intensive housing, meanwhile Mangalicas were individually penned in outdoor system. All animals were fed according to their genotype and age.

The CT scanning of the pigs was performed using a Siemens Somatom S40 equipment at the Institute of Diagnostic Imaging and Radiation Oncology of the Kaposvár University. The details of the applied premedication and inhalation anaesthesia were already published (*Petrási et al*., 2001). The data collection was performed in spiral mode. Consecutive cross-sectional scans of 10 mm slice thickness were reconstructed from the raw data covering the whole body. The picture-forming pixels of the scans are in fact small prisms (voxel) with definite volume (10 mm^3). It is possible, therefore, to determine the part of the total volume of the examined scan that falls into the Hounsfield unit (HU) interval of interest. This enabled us to estimate the volume of different tissues of the body from serial scans (*Romvári et al*., 1996). By the image evaluation from the total Hounsfield scale only 400 density values, ranging from –200 to +200, were taken into account, belonging to fat, and muscle tissue (water = 0). From these values all 10 neighbouring ones were summarised, resulting in altogether 40 Hounsfield variables (HUv) (*Romvári et al*., 1998).

The tissue composition and its changes were demonstrated by means of histograms. Using volumetric estimation, the total muscle percentage (sum HUv22- HUv40 / HUv1-HUv40 x 100), and the muscle to fat ratio (HUv22- HUv40 / HUv1- HUv18) was determined (without intestines). Moreover, on the cross-sectional images the cross-sectional area and the average X-ray density of *m. longissimus dorsi* and *m. semitendinosus* were calculated at the kidneys and at the knee joint, respectively. Furthermore, the fat thickness was also measured. Finally, twenty-five scans representing identical anatomic points (from the neck (atlas) to the hock) were used for the graphical demonstration of the body composition. Based on the 40 HUv, 3D histograms were developed with the method of the negative exponential interpolation to characterise the amount of fat and muscle tissue of the body at the live weight of 30 and 90 kg.

RESULTS AND DISCUSSION

In order to determine the tissue composition of the meat-type and Mangalica pigs, X-ray density distributions are depicted (*Figures 1-2*), where the frequency of voxels are illustrated on the Y-axis and the HU variables (ranging from –200 to +200) on the X-axis. Concerning meat-type pigs, clear peaks can seen at the section of the X-axes corresponding to muscle (HU22-HU40) , while less pronounced peaks were found at the density values related to fat (HU1-HU18) (*Figure 1*). Muscle content was substantial at all times of investigation showing intensive deposition. Fat ratio between the weight range of 30–90 kg was smaller. This tendency was the same for all weight categories. Based on the volumetric data, the calculated muscle and fat volumes were 15.5, 24.8, 35.2 and 4.6, 7.8, 17.6 dm^3, respectively in the average weight of 30, 60 and 90 kg. Similarly measured values of the Mangalica pigs can be viewed at *Figure 2*.

Figure 1

Histograms of meat-type pigs in the -200 to +200 Hounsfield interval (1–40 HU variables)

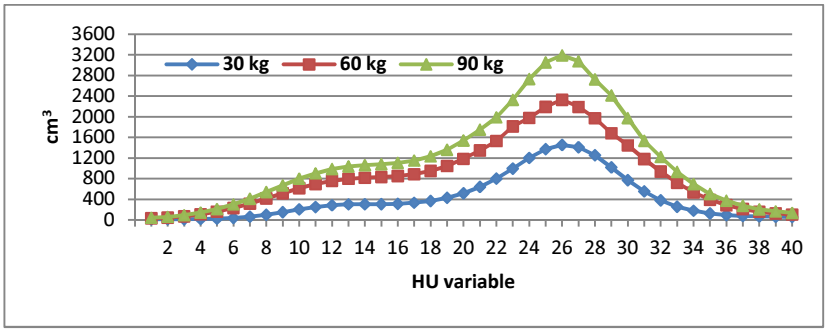
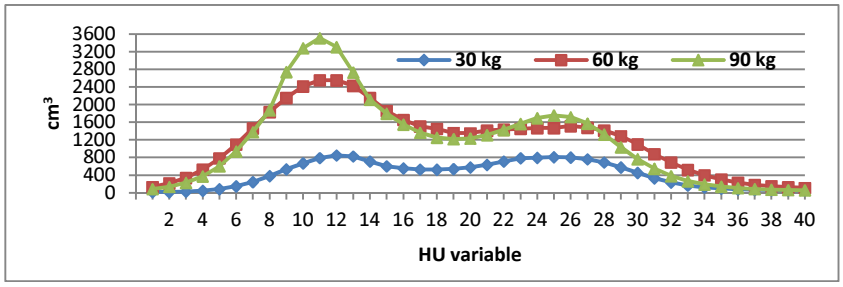


Figure 2

Histograms of mangalica pigs in the -200 to +200 Hounsfield interval (1–40 HU variable)



Mangalica pigs weighing 30 kg showed approximately identical peaks related to muscle and fat, respectively. At the second time of investigation of these animals weighing 60 kg, an intensive fat deposition was observed coupled with a less intensive lean gain. At

the body weight of 90 kg no lean tissue gain could be found, however further substantial increase of the fat ratio was detected. The concerning muscle and fat volume data were 8.6, 15.1, 17.9 and 8.9, 27.3, 37.4 dm^3, respectively, in the three weight categories.

On the 3D histograms the serial number of the pictures was illustrated on the X-axis, the HU variables on the Y-axis (numbering from 40 to 1 after reducing by 10 from + 200 to - 200) and the frequency of the density values on the Z-axis. For the comparability of the histograms prepared from the different genotypes identical scaling was used. In the case of the 30 kg animals, two characteristic peaks can be clearly recognized in the muscle tissue interval (HUv 21–40). The first from the head is the periphery of the scapular arch (2–8). The next, something lower part is the spine, after which the highest "peak", formed by the ham can be seen (18–25). In the fat interval (HUv 2–18) remarkable differences can be seen within the two genotypes, especially in the abdominal region (*Figure 3*). According to the 3D surface and volumetric data the Mangalica can be characterized with equal volume of fat and muscle tissue in the examined liveweight.

The tissue distribution in 90 kg are very similar to that of the animals weighing 30 kg. The earlier described differences of the two genotypes became stronger as the meat-type and the Mangalica pigs showed an intensive muscle and fat tissue deposition (*Figure 4*).

Figure 3

3D histograms of meat-type (left) and Mangalica pig (right) at 30 kg

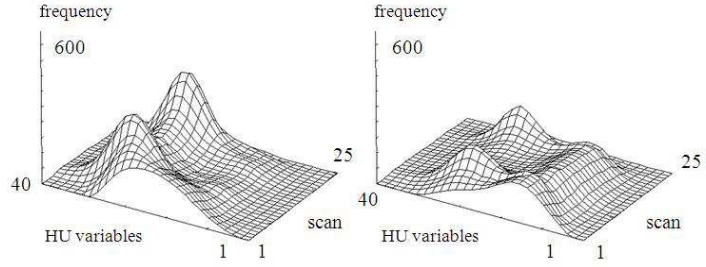
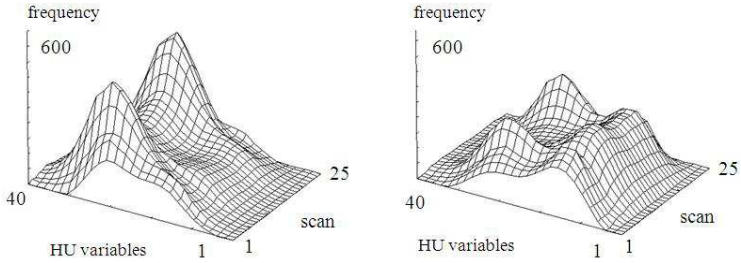


Figure 4

3D histograms of meat-type (left) and Mangalica pig (right) at 90 kg



Examining the tissue composition of the analysed meat- and fat-type pigs opposite muscle to fat ratio was found. Having the same body weight, the muscle content of the meat-type pigs was almost identical with the fat content of the Mangalicas. At 30 kg, being the first stage of our investigation, the lean meat percentages of 61.5% and 35.5% were recorded on the meat-type and on the Mangalica pigs, respectively, showing 3.4 and 1.0 muscle to fat ratios. Based on the CT scanning a dominant lean gain and fat deposition was found on the meat-type and Mangalica pigs, coupled with an increasing body weight. At 60 kg body weight the lean meat percentage of the former group showed 57.7% lean meat and 3.2 muscle to fat ratio, while the related values of the latter group were 30.9% and 0.6, respectively. At the final stage of investigation (90 kg), practically no muscle deposition was found in the Mangalica pigs while an increase in the fat tissue volume could be detected, compared to the preceding time of investigation. At this specific time their lean meat percentage and muscle to fat ratio decreased to 28.5% and 0.5, respectively. Compared to the data measured at the live weight of 60 kg, further intensive growth was found by the meat-type pigs, resulting in a lean meat percentage and muscle to fat ratio of 53.2% and 1.9, respectively.

Figure 5

CT images at the level of the kidney of meat- (left) and fat-type (right) pig at 90 kg

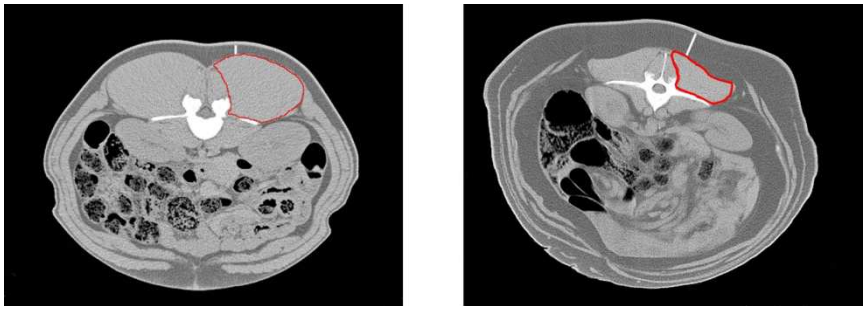
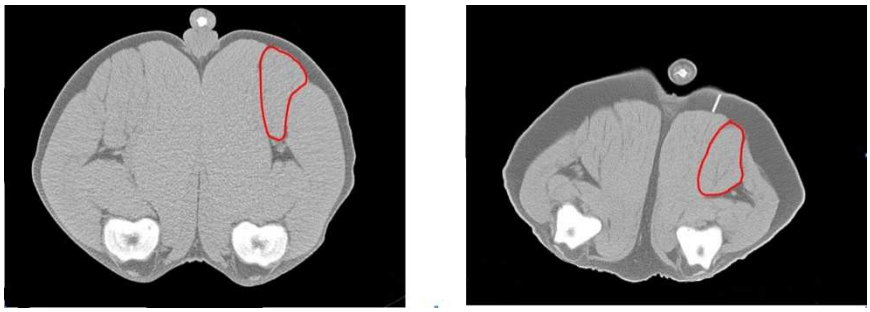


Figure 6

CT images at the level of the knee joint of meat- (left) and fat-type (right) pig at 90 kg



At the live weight of 90 kg basic anatomical differences of the two examined genotypes can be seen on *Figures 5.* and *6.* showing cross-sectional images at the level of the kidney, and ham (and knee joint, resulted from the stretched hind limbs). Extreme differences between the meat- and fat-type pigs are clearly visible in the images. The average cross sectional surface values of the right *m. longissimus dorsi* were determined, which showed 45 and 23 cm^2 in the case of the meat- and fat-type pigs, respectively, verifying the reported results (38.4 and 20.9 cm^2) (12). The concerning values of *m. semitendinosus* were 31 and 24 cm^2 at the liveweight of 90 kg, respectively.

Besides the loin area, fat depth values were also provided by the CT images measured 8 cm laterally from the spine. At the areas of kidney and rump the recorded fat thickness values were 53, 38 and 18, 16 mm on average on the Mangalica and meat-type pigs, respectively. The previously mentioned authors recorded fat depth values of 48, 58 mm on Mangalicas and 28, 38 mm on meat-type pigs. The former values were rather similar to those found in the present investigation but the latter values differed significantly as a result of changes took place during the past approximately 20 years. Using the same CT images as before, frequency curves of the loin and ham were depicted in order to quantitatively analyse IMF content on pigs weighing 30 and 90 kg, using average X-ray density values. Concerning meat-type pigs 53 and 58 HU were found on the loin meanwhile 52 HU were recorded on the ham at both (consecutive) times of investigation. Average density values of 46, 47 and 32, 34 were obtained on the loin and ham of Mangalica pigs. The latter results of both muscles demonstrate that intramuscaular fat of the Mangalica pigs – especially on the ham – exceeds that of the meat-type pigs causing lower HU variables. Similar findings were described about the IMF content comparing four genotypes including F2 Mangalica pigs (*Kipfmuller et al*., 2000).

CONCLUSIONS

The phenomenon that potential daily lean tissue growth of meat-type pigs can be almost constant for most of the fattening period was justified by the received results (*Whittemore*, 1986). The growing intensity and capacity of lean tissue of the Mangalica pigs was low, while the fat deposition was significant during the whole period of the investigation. At the body weight of 60 kg, the lean gain was practically stopped, while the fat deposition continued on considerably. The applied negative exponential interpolation presented through 3D histograms is a clearly suitable method for describing growth characteristics and tissue deposition differences of different genotypes as demonstrated on rabbits (*Romvári et al*., 1998), turkey (*Andrássy-Baka et al*., 2003), and fish (*Hancz et al*., 2003). Regarding Mangalica pigs (contrary to meat-type conspecies), our results clearly demonstrated the gain of substantial amount of IMF during the growing period also showing significantly higher fat thickness values than that of the meat-type pigs, verifying the results based on test slaughters (*Adilovic et al*., 1985; *Rede et al*., 1986). In our opinion the *in vivo* CT scanning is an appropriate method to study the IMF content with certain limitation, namely the sensitivity of the process is increasing with the increasing IMF content.

The applied non invasive procedures are appropriate to monitor the changes of body composition of extremely different pig genotypes during the growing period and also the selection-induced alterations are clearly demonstrated within the species. The *in vivo* determination of body composition, particularly lean and fat content, can provide an effective tool to be used in the selection.

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The effect of lairage time and carcass traits of pigs on the appearance of subcutaneous veining defect in hams

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ABSTRACT

The incidence of veining defect was quantified on 1,919 pigs slaughtered in three consecutive days in one commercial slaughterhouse in Slovenia. Veining defect was evaluated after rapid chilling with subjective method proposed by Russo et al. (2003). Hams were graded into four classes, from none to heavy defect. Around 30% hams belonged to class 1, 60% to class 2 and 6% to class 3. There was practically no ham in class 4. Prolonged lairage time (more than 3 h) increased the incidence of veining defect. Hanging carcass on one foot during exsanguination also increased veining defect compared to the counterpart. Carcass weight increase from 60 to 100 kg also increased the incidence of veining defect, while further increase of carcass weight had no effect on veining defect. With increased muscle thickness, decreased fat thickness and increased lean meat percentage the incidence of veining defect also increased.
(Keywords: pigs, lairage time, carcass traits, veining defect)

INTRODUCTION

Dry-cured ham "Kraški pršut" is one of the most well-known and appreciated meat products in Slovenia. To achieve high quality product it is important to use only hams with certain characteristics (*Čandek-Potokar and Škrlep*, 2012). Visual appearance is very important and only hams without any visual defects are appropriate. Veining defect is one of the visual defects representing a clearly visible network of subcutaneous blood vessels (*Russo et al*. 2003). The reasons for this defect are poorly understood. *Russo et al.* (2003) reported increased incidence of veinig defect with increased lean meat percentage and prolonged lairage time in Italian heavy pigs. On the other hand Nanni-*Costa et al*. (2005) found no difference in the incidence of veining defect in pigs resting 0 or 24 h before slaughter. *Lo Fiego et al.* (2005) reported the effect of stunning method on the incidence of veining defect. CO_2 stunned pigs exhibited more pronounced veining defect than the electrically stunned ones. If this effect becomes more pronounced, such hams cannot be used for dry-cured ham.

The aim of this study was to get the first insight into the incidence of this defect in pigs slaughtered in Slovenia and to investigate the effect of lairage time and some carcass traits (carcass weight, muscular and fat thickness) on the incidence of subcutaneous veining defect.

MATERIAL AND METHODS

In three consecutive days 1.919 pigs from 17 different farms, slaughtered in the same slaughterhouse were used to evaluate lairage time and carcass traits on the appearance of veining defect. After unloading, pigs spent different time in lairage and were divided into four classes (class 1 lairage time ≤30 min; class 2 lairage time >30 ≤60 min; class 3 lairage time >60≤180 min; and class 4 lairage time > 180 min. Pigs have been subjected to CO_2 stunning (80% CO_2), hung on the left foot and exsanguinated. About 30 min after exsanguination carcasses were graded according to SEUROP system based on carcass weight, muscle and fat thickness (*Pravilnik*, 2004). Muscle thickness is defined as a distance in mm between the cranial edge of *m. gluteus medius* and the dorsal edge of *canalis vertebralis* measured at the carcass split line, whereas the thinnest part in mm of fat thickness is measured at the level of *m. gluteus medius* at the carcass split line. After grading rapid chilling began and lasted for 140 min. In this period the carcasses were first exposed to -2 °C, then -15 °C, -12 °C and -8 °C. At the end of rapid chilling the left and right hams were subjectively examined for veining defect, using the evaluation scale of 4 classes (1=no defect or barely observable, 2=light, 3= evident, 4= heavy) according to *Russo et al*. (2003). Data were processed by the NPAR1WAY procedure and WILCOXON test of *SAS* (2003) using a non-parametric model which included lairage time, left or right ham, carcass weight, muscle thickness or fat thickness as a single factor. The frequency distribution of hams into four classes was calculated for each treatment. According to carcass weight, muscle thickness and fat thickness the animals were divided into seven classes (carcass weight: class 1 CW≤60 kg; class 2 CW>60≤70 kg; class 3 CW>70 ≤80 kg; class 4 CW>80≤90 kg; class 5 CW>90 ≤100 kg; class 6 CW>100 ≤110 kg; class 7 CW>110 kg) and four classes (muscle thickness M: class 1 M≤60 mm; class 2 M>60 ≤70 mm; class 3 M>70≤80 mm and class 4 M>80 mm and fat thickness F: class 1 F≤10 mm; class 2 F>10 ≤15 mm; class 3 F>15≤20 mm; class 4 F>20 mm and lean meat percentage LM%: class S LM%≥ 60%; class E LM%≥55<60%; class U LM%≥50< 55% and class R LM%≥50<45 %).)

RESULTS AND DISCUSSION

In *Table 1* the average incidence of veining defect is presented. Most of the hams were classified into the second class with light observable defect (63.4%), followed by class 1 with no or barely observable defect (30.4%) and class 3 with evident veining defect (6.2%). *Russo et al.* (2003) found slightly higher percentage of hams in class 1, lower percentage in class 2 and similar percentage in class 3. Also *Lo Fiego et al*. (2003) found similar percentage of hams in class 1 and 2 at electrically stunned pigs. The most important difference is between class 2 and 3, as hams classified in class 3 are not suitable for dry-cured ham (*Russo et al*., 2003).

Table 1

Distribution of hams (%) into different classes of veining defect

Total number of hams	Veining defect			
n	Class 1	Class 2	Class 3	Class 4
3838	30.4	63.4	6.2	0.0

Lairage time had a significant effect on the incidence on veining defect, with prolonged lairage time the veining defect increased too (*Table 2*). The percentage of hams graded in class one decreased whereas the percentage of hams in class 2 and 3 increased. Especially when pigs stayed in lairage longer than 180 min, the percentage of hams in class 3 more than doubled. This is in contrast with the results of *Nanni Costa et al.* (2005), who did not find any difference in veining defect in Italian heavy pigs slaughtered immediately or after 24 h.

Table 2

Distribution of hams (%) into different classes of veining defect in relation to lairage time*

		Veining defect			
Lairage time	**n**	**Class 1**	**Class 2**	**Class 3**	**Class 4**
≤30 min	1633	38.1	58.0	3.9	0
>30 ≤60 min	874	27.1	67.8	5.0	0
>60 ≤180 min	976	25.0	67.4	7.5	0.1
>180 min	329	13.4	69.6	17.0	0

* X^2 Significant effect of lairage time p value < 0.0001

The incidence of veining defect also increased with increased carcass weight to 100 kg (*Table 3*). Afterward the percentage of hams in class 1 did not change, whereas the percentage of hams in class 3 even decreased slightly due to the increased percentage of hams in class 2.

Table 3

Distribution of hams (%) into different classes of veining defect in relation to carcass weight*

		Veining defect			
Carcass weight	**n**	**Class 1**	**Class 2**	**Class 3**	**Class 4**
≤60 kg	46	34.8	65.2	0	0
>60 ≤70 kg	320	39.4	57.8	2.8	0
>70 ≤80 kg	844	32.6	63.3	4.1	0
>80 ≤90 kg	1349	29.9	63.7	6.4	0
>90 ≤100 kg	892	26.0	65.1	8.7	0.1
>100 ≤110 kg	311	26.4	65.3	8.4	0
>110 kg	50	26.0	68.0	6.0	0

* X^2 Significant effect of carcass weight p value < 0.0001

Muscle thickness significantly affected the incidence of veining defect (*Table 4*). With increased muscle thickness the percentage of hams in class 1 decreased whereas the percentage of hams in class 2 and 3 increased. When the muscle thickness increased from 60–70 mm to more than 80 mm, the percentage of hams in class 3 more than triplicated.

Table 4

Distribution of hams (%) into different classes of veining defect in relation to muscle thickness*

		Veining defect			
Muscle thickness	**n**	**Class 1**	**Class 2**	**Class 3**	**Class 4**
≤ 60 mm	101	47.5	49.5	3.0	0
> 60 ≤ 70 mm	1174	34.1	62.7	3.2	0
> 70 ≤ 80 mm	2053	27.9	65.0	7.1	0
> 80 mm	484	26.2	63.4	10.3	0

* X^2 Significant effect of muscle thickness p value <0.0001

Fat thickness had also a significant effect on the incidence of veining effect (*Table 5*). It was at least pronounced when fat thickness exceeded 20 mm and the percentage of hams in class 3 almost halved.

Table 5

Distribution of hams (%) into different classes of veining defect in relation to fat thickness*

		Veining defect			
Fat thickness	**n**	**Class 1**	**Class 2**	**Class 3**	**Class 4**
≤10 mm	656	34.3	59.0	6.7	0
>10≤15 mm	1852	30.5	62.8	6.6	0
>15≤20 mm	951	26.3	67.5	6.1	0.1
>20 mm	353	30.3	66.3	3.4	0

* X^2 Significant effect of fat thickness p value < 0.025

The muscle and fat thickness and carcass weight are used to calculate the lean meat percentage in Slovenia. Increased muscle thickness and decreased fat thickness is correlated with increased lean meat percentage in pig carcass. The effect of lean meat percentage on the incidence of veining defect is well seen in *Table 6*. As lean meat percentage increased, the incidence of veining defect increased too. There was no veining defect present in class R, whereas in class U and E 2.5. and 6.3 pigs exhibited evident veining defect. *Russo et al.* (2003) reported higher incidence of veining defect in pigs with higher lean meat percentage in the carcass. Classes 3 and 4 almost doubled when the lean meat percentage in the carcass increased from less than 47% to more than 49 % in Italian heavy pigs.

During exsanguination the pigs were hung on left foot. From *Table 7* it is clear that the incidence of veining defect was higher on left ham. So it seems the additional tension that the left ham was exposed during the exsanguination had also an effect on the incidence of veining defect.

Table 6

Distribution of hams (%) into different classes of veining defect in relation to SEUROP classification*

SEUROP class	n	Veining defect			
		Class 1	Class 2	Class 3	Class 4
S	1850	31.1	61.8	7.1	0
E	1549	28.7	65.0	6.3	0.1
U	357	28.3	69.2	2.5	0
R	36	52.8	47.2	0	0

* X^2 Significant effect of EUROP classification p value < 0.0097

Table 7

Distribution of hams (%) into different classes of veining defect in relation to left/right ham*

		Veining defect			
	n	Class 1	Class 2	Class 3	Class 4
Left**	1892	28.5	64.4	7.1	0.0
Right	1892	31.7	62.9	5.3	0.0

* X^2 Significant effect of left/right ham p value < 0.0095
** hung on left foot

CONCLUSIONS

From this first survey of the incidence of veining defect in Slovenian pig population it is clear that this problem has a similar extent as in Italian pigs. Keeping lairage time shorter than 3 h can contribute to lower incidence of veining defect. With increased lean meat percentage in carcass and also in ham, the quality of hams for dry-cured ham decreased, so the balance between this two groups of traits should be preserved.

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The effect of Se-fortified wheat in feed on concentrations of selenium and GPx and SOD in blood of laying hens

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ABSTRACT

The aim of the study was to determine concentrations of selenium and enzymes GPx and SOD in blood of hens which were consuming feed with Se-fortified wheat. The study was conducted on 70 Tetra SL hybrid hens which were in the 40th week of production. Hens were fed with prepared mixtures for 26 days. After this period hens' blood was sampled for analysis of selenium and glutathione peroxidase (GPx) and superoxide dismutase (SOD) content. Treatments used in the study had no effect on weight of hens and egg production between examined groups (P>0.05). Selenium content in laying hens' blood of both groups was balanced and amounted for group A=0.1878 µg/kg and for group B=0.1877 µg/kg (P>0.05). The higher activity of the GPx enzyme was determined in blood of hens from experimental group B compared to group A (42935.3 U/l and 35675.5 U/l, respectively, P<0.05). SOD values showed similar trend as GPx values, with significantly higher (P<0.01) activity of this enzyme in blood of group B hens (0.9955 U/l) compared to group A hens (0.8101 U/l). Using wheat fortified with selenium in the diets for laying hens can affect a better supply of this microelement what was determined from the blood analysis.

(Keywords: wheat, laying hens, selenium, blood, enzymes)

INTRODUCTION

Selenium is an essential trace element for people and animals and it have to be taken with food. Selenium have multiple role in many biochemical processes in the body. Sufficient body supply with selenium will affect a better condition of immune, enzymatic and reproductive system (*Surai,* 2002). Selenium is a major component of the antioxidant defense mechanism in all living tissues because it is an integral part of a number of enzymes involved in cellular antioxidant defense (*Tapiero et al.*, 2003). Antioxidant system at the cellular level consists of three levels of defense. The first level of defense is responsible for preventing the formation of free radicals by removing their precursors. Defense at this level is provided by superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione, thioredoxin system and metals that make up proteins. The second level of defense is responsible for the prevention of chain breaking and its propagation, and it consists of vitamins A, C and E, carotenoids, ubiquinones, glutathione and uric acid. Lipases, peptidases, proteases, transferases, enzymes for DNA repair are parts of the third level of cellular protection and are responsible for cutting and repairing damaged parts of the molecule (*Surai,* 2006). Selenium is added to the poultry feed in two forms, inorganic and organic. A lot of

researchers have described better bioavailability of selenium if it is added to the feed in organic form (*Skrivan et al*., 2006; *Payne et al*., 2005; *Rayman*, 2004). Crop production aims to increase content of various trace elements in plants through fortification, which will then be consumed by animals in their diet in organic form.

The aim of this study was to determine concentrations of selenium and enzymes GPx and SOD in blood of hens that were fed mixtures with Se-fortified wheat.

MATERIAL AND METHODS

The study was conducted on 70 Tetra SL hybrid hens, divided into two groups (A and B). Hens were in the 40th week of production and during 26 days they were consuming mixtures in which 10% of corn was replaced with wheat. Wheat of Srpanjka variety was grown on calcareous soils with 7 different treatments in a randomized block design, and for feeding experiments wheat from two fertilization treatments was used, as follows: A=control without Se and B=foliar application of Se (10 g Se ha^{-1}). The composition of mixtures for hens is shown in *Table 1*. Analysis of selenium content in mixtures was made shortly before feeding trial. It was determined that mixture A contains 0.3059 mg Se/kg diet, and mixture B 0.5484 mg Se/kg diet. During the experimental period production of eggs was recorded, and weight of hens was controlled (beginning and end of the experiment).

At the end of the trial period 7 animals from each group were randomly selected for blood drawning in order to determine the concentration of selenium and status of enzymes glutathione peroxidase (GPx) and superoxide dismutase (SOD). Blood was drawn from the wing vein into sterile vacuum tubes BD Microtainer® SST™ (Becton, Dickinson and Company, USA). GPx concentration was determined from the whole blood and SOD concentration from serum of same animals. For enzyme determination commercial kits Randox Ransel RS 505 and Randox Ransod SD 125 (Laboratories Ltd, London, UK) were used, while selenium concentration in the blood was analyzed using a Perkin Elmer Optima 2100 DV device (*Davidowski*, 1993). Results of the research were analyzed by statistical software Statistica for Windows version 12.0 (StatSoft Inc., 2013).

RESULTS AND DISCUSSION

Table 2 shows the weight of laying hens used in the experiment, at the beginning and at the end of the experiment, and the production of eggs during the trial period. Treatments used in the experiment had no effect on the difference in weight of hens and egg production between two groups (P>0.05). In a study on the influence of fortified barley on performance of laying hens and their offspring *Hassan* (1990) reported that concentrations of selenium in fortified barley has no effect on the weight of laying hens, which is consistent with our results. *Haug et al.* (2008) in the study on the bioavailability of selenium from feed that contains wheat fortified with selenium on the performance and selenium content in muscle tissue of chickens, pointed out that the levels of selenium in feed had no effect on the weight of chickens, which is in accordance with our research. *Gjorgovska et al*. (2012) found statistically significant effect of organic selenium levels in feed for laying hens on egg production. Their results are not consistent with ours. *Yoon et al.* (2007) reported results consistent with ours and pointed out that the source but also the level of selenium in feed has no effect on the weight of chickens (P>0.05).

Table 1

Composition and chemical analysis of the mixture for laying hens

Ingredient, %	**[1]A and B**
Corn	40,75
Triticale	6,60
Wheat	10,00
Soybean meal	18,33
Toasted soy	8,33
Sunflower meal	1,66
Alfalfa	1,00
Calcium granules	8,13
Monocalcium	1,58
Yeast	0,50
Salt	0,33
Mineral Detox	0,25
Probiotic Pro Bio	0,05
Methionine	0,25
Premix[2]	0,58
Soybean oil	1,66
Total	100,00
[3]Chemical analysis of the mixture (g/kg)	
Moisture	87
Crude protein	190
Crude fiber	40
Ash	139
Fat	52
Ca	41

[1]in mixtures 10% of corn was replaced with wheat as follows: A=control without selenium fortification and B=wheat fortified with 10 g Se ha^{-1}

[2]Premix smixture K, content in 1 kg: vitamin A 200000 UI, vitamin D_3 500000 UI, vitamin E 10000 mg, vitamin K_3 600 mg, vitamin B_1 400 mg, vitamin B_2 1000 mg, vitamin B_6 1000 mg, vitamin B_{12} 3000 µg, vitamin C 4000 mg, vitamin H 12 mg, vitamin B_3 8000 mg, vitamin B_5 2400 mg, vitamin B_9 150 mg, vitamin B_4 100000 mg, iodine 200 mg, manganese 18000 mg, zinc 14000 mg, cobalt 30 mg, iron 12000mg, copper 1600 mg, inorganic selenium 50 mg, calcium 238 g, phytase 100000 FYT, canthaxanthin 500 mg, beta-apo-beta-carotenoic acid 300 mg, antioxidant (butyl hydroxytoluene) 20000 mg

[3]Reference methods used for chemical analysis of feed: HRN ISO 6496:200; HRN EN ISO 5983-2:2010; HRN EN ISO 6865:2001, modified according to the instructions FOSS Fiber Cap manual; HRN ISO 5984:2004; HRN ISO 6492:2001, modified according to the instructions of the extraction system ANKOM XT15; RU-5.4.2-11 (internal method)

Stressful situations in mammals, such as starvation and intensive production, are associated with changes in the antioxidant defense of the body and it can vary according to the species, while values in the blood are not correlated with those determined in the organs (*Wohaieb and Godin*, 1987), because of particular role of the metabolism of certain organs. Unlike mammals, birds have a higher body temperature and more intense metabolism and therefore they need much more oxygen. For this reason birds are exposed to stronger oxidative stress. Adding selenium to poultry feed in intensive farming has a positive effect on the increase in antioxidant activity.

Table 2

Weight of hens and egg production

Production indicators Hens weight (g)	A ($\bar{x}$ ±sd)	B ($\bar{x}$ ±sd)
Experiment beginning	2081,60±144,82	2062,68±133,65
Experiment end	2159,91±230,38	2083,14±129,66
Egg production (pcs.)	882	880

Table 3 shows the effect of selenium levels in feed for laying hens on concentration of selenium and enzymes GPx and SOD in blood. It is evident that the level of selenium in mixtures for laying hens has no effect on the concentration of selenium in the blood (P>0.05). A higher concentration of the enzyme GPx was found in hens in group B compared to group A (42935.3 U/l and 35675.5 U/l, respectively, P<0.05). SOD values showed similar trend as GPx values, with significantly higher (P<0.01) values of this enzyme in the blood of group B hens (0.9955 U/l) compared to group A hens (0.8101 U/l). *Gajčević et al.* (2009.) point out that higher level of selenium in the diet (0.4 ppm) has a positive effect on GPx activity in blood of laying hens (P<0.05). Numerous studies related to the use of selenium in poultry feed represent a connection to research of the impact of selenium sources (inorganic or organic) on different production traits in animals. At the conclusion of each of the studies better results were obtained using an organic form of selenium. *Mahmound and Edens* (2003), who were investigating the impact of different sources of selenium on the biochemical changes in the blood of hens, found a higher GPx activity in the blood of birds that consumed selenium from organic sources in the diet. Positive experiences of using organic selenium compared to inorganic selenium in poultry feed mentioned also *Leng et al.* (2003), who highlighted the positive correlation between levels of selenium in feed and GPx activity in blood of broiler chickens.

Table 3

Influence of selenium level in feed on concentrations of selenium and GPx and SOD enzymes in blood of laying hens

Indicator	A ($\bar{x}$ ±sd)	B ($\bar{x}$ ±sd)
Se (µg/kg)	0,1878±0,008	0,1877±0,006
GPx (U/l)	$35675,5\pm5713,4^{b}$	$42935,3\pm3589,7^{a}$
SOD (U/l)	$0,8101\pm0,088^{B}$	$0,9955\pm0,058^{A}$

Values in rows marked with ^{A,B} exponents differ at P<0,001 level, and those marked with ^{a,b} differ at P<0,05 level.

Hassan (1990) stated that activity of GPx enzyme was significantly reduced (P<0.01) in hens with a deficit of selenium in feed. In accordance with our results *Yoon et al.* (2007) state that increased levels of organic selenium in feed can significantly (P<0.05) increase concentration of selenium and GPx enzymes in the blood of chickens.

CONCLUSIONS

From the research results it can be concluded that the use of Se-fortified wheat in feed for laying hens influenced greater supply of body with selenium, which resulted in significantly higher levels of GPx and SOD enzymes in blood of hens from experimental group B compared with group A ($P<0.05$ and $P<0.01$). In order to obtain even better results it is necessary to expand the research, and to add fortified corn as well as a greater proportion of fortified wheat into laying hens' feed.

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Effect of storage period on the quality of table eggs

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ABSTRACT

The research aim was to determine effect of storage period (measured on the 1st day and 28th day) on the quality of conventional table eggs and omega-3 eggs. The research was carried out on 120 eggs, of which 60 were conventional and 60 were produced within omega-3 production system. Research results showed that the storage period of conventional eggs significantly affected (P<0.05) increase of albumen and yolk pH, as well as aging rate, while reducing the values of albumen height, HU, yolk color and value number. Analysis of external and internal quality of omega-3 eggs indicated that the storage period significantly affected (P<0.05) increase of albumen and yolk pH and aging rate, and reduced values for egg shell strength and thickness, albumen height, HU, and value number of stored eggs. Lipid oxidation was significantly higher in eggs stored for 28 days at 4 °C if compared to fresh eggs (P<0.05).
(Keywords: quality, eggs, omega-3, TBARS)

INTRODUCTION

Changes in yolk and albumen occur during storage of eggs, initiating hydrolytic process of protein degradation and lipid degradation. *Gajčević* (2010) stated that lipid peroxidation referred to oxidative degradation of lipids in cells. Lipid peroxidation is catalyzed by hemic and non-hemic iron. Dissociation of LOOH affects accumulation of short-chain final peroxidation products, such as aldehydes and hydrocarbons, which are responsible for unpleasant odor of oxidized fat (*Adams*, 2003). Lipids consist of different molecules, such as triglycerides, free fatty acids, xanthophylls, carotenes, vitamins and phospholipids. Their common characteristic is a molecule with a long chain of carbon atoms being connected with many double bonds. These double bonds between atoms make lipids extremely sensitive to oxidation. In the process of oxidation, lipids lose some nutritive characteristics. Fats and oils become rancid, vitamins reduce their biological value and pigments lose color. This affects the decrease of nutritive value and sensory characteristics of a product.

The aim of our research was to investigate effect of storage period (1st and 28th day) on qualitative indicators of conventional and omega-3 eggs.

MATERIAL AND METHODS

Omega-3 eggs (n=60) and conventional eggs (n=60) were used for the purpose of analyzing effect of storage period on quality of table eggs. Quality of eggs was determined one day after collecting eggs and on the 28th day of storing eggs in refrigerator at +4°C. The research was conducted on Tetra SL genotype hens which were in the 40th week of production. Hens were fed with different mixtures, one group with

conventional feed and another with modified feed (which contains 5% oil mixture – soybean, rapeseed, linseed and fish oil). Analyzed indicators of external quality of eggs were: egg weight, shell strength and thickness, shell weight. Analyzed internal egg quality indicators were: weight of albumen and yolk, yolk color, albumen height, Haugh units, pH of albumen and pH of yolk, refraction of albumen and yolk, which values were used for calculation of value number (VN) and aging rate (AR) by the following expressions: $VN = 1000 \cdot (\eta_ž - \eta_b)$; where $\eta_ž$= *yolk refraction index*; η_b = *albumen refraction index* and $AR = 1000 \cdot (1{,}4184 - \eta_ž)$; where 1.4184=*fracture index of standard yolk-referential value*; $\eta_ž$= *yolk refraction index* (*Janke and Jirka*, 1934).

Egg shell strength was measured by the Eggshell Force Gauge Model-II device. Thickness of shell was measured in its middle by electronic micrometer, precision of 0.001 mm. Yolk color, HU and albumen height were measured by the Egg Multi-Tester EMT-5200 device. Albumen and yolk pH value were measured by the pH meter MP 120, refraction of albumen and yolk was measured by automatic device Refracto 30PX. Lipid oxidation was determined on 40 yolks (20 fresh and 20 stored), according to modified method of *McDonald and Hultin* (1987) and *Botsoglou et al.* (1994). Yolks weighed into a test tube were mixed with 10% trichloroacetic acid (1w:3v), homogenized and centrifuged at 10000 rpm for 10 minutes at 4 °C. Supernatant was then mixed with solution of thiobarbituric acid. Tubes were closed and placed into a water bath at 90 °C for 30 minutes. Distilled water was added after cooling and mixture was then centrifuged at 6000 rpm for 5 minutes at 4 °C. The content of colored product that occurred as a reaction of lipid peroxidation with thiobarbituric acid was measured spectrophotometric at 534 nm. Obtained results of all analyses on eggs were processed in Statistica 7.1 (*StatSoft, Inc*., 2007), and presented in tables and graphs along with discussions and conclusions. Statistical indicators referred to arithmetic mean ($\bar{x}$) and error of the mean ($s_{\bar{x}}$). Testing of significance of differences for egg quality was done by the t-test. Calculated values were compared with theoretical value at a significance level of $P<0.05$.

RESULTS AND DISCUSSION

Table 1 overviews results related to weights of conventional eggs and their main parts, both fresh and stored for 28 days at +4 °C. As presented, storage period had effect ($P<0.05$) on egg weight (67.46 g and 65.85 g) and albumen weight (41.12 g and 39.96 g), while values of yolk weight (15.53 g and 15.78 g) and shell weight (7.68 g and 7.61 g) for both fresh and stored eggs were similar ($P>0.05$).

Table 1

Weight of conventional eggs and main parts ($\bar{x} \pm s_{\bar{x}}$)

Indicator, g	Fresh (n=30)	Stored* (n=30)
Weight of eggs	67.46 ± 0.44^a	65.85 ± 0.43^b
Weight of albumen	41.12 ± 0.30^a	39.96 ± 0.29^b
Weight of yolk	17.75 ± 0.21	17.54 ± 0.25
Weight of shell	8.58 ± 0.11	8.29 ± 0.11

*28 days in refrigerator at +4 °C; $\bar{x}$ = mean value; $s_{\bar{x}}$= error of the mean; numbers in rows marked ^{a,b}differ statistically ($P<0.05$)

Table 2 shows results of storage period affecting external and internal quality of conventional eggs. It is obvious that storage period did not affect strength and thickness of shell in conventional eggs (P>0.05). However, storage period of conventional eggs had statistically significant (P<0.05) effect on values albumen height, HU, yolk color and VN. Values of these indicators were reducing along with storage duration. Albumen height reduced from 6.94 mm to 6.16 mm, HU reduced from 80.80 to 75.06, yolk color reduced from 8.70 to 7.86, and VN from 63.26 to 60.50. Conventional eggs exhibited statistically significant effect of storage period on the increase of pH in albumen, pH in yolk and AR. Values of pH in albumen increased from 8.66 to 9.01, pH in yolk increased from 5.99 to 6.06. AR increased from 0.933 to 1.90.

Table 2

External and internal quality indicators conventional eggs ($\bar{x} \pm s_{\bar{x}}$)

Indicator	Fresh (n=30)	Stored* (n=30)
Strength of shell, kg/cm^2	3.100±0.11	3.160±0.10
Thickness of shell, mm	0.409±0.007	0.406±0.005
Albumen height, mm	6.94±0.17^a	6.16±0.23^b
Haugh units	80.80±1.16^a	75.06±1.89^b
Yolk color	8.70±0.08^a	7.86±0.10^b
pH of albumen	8.66±0.02^b	9.01±0.01^a
pH of yolk	5.99±0.01^b	6.06±0.01^a
AR	0.933±0.20^b	1.90±0.27^a
VN	63.26±0.29^a	60.5±0.85^b

*28 days in refrigerator at +4 °C; $\bar{x}$ = mean value; $s_{\bar{x}}$= error of the mean; numbers in rows marked ^{a,b}differ statistically (P<0.05)

Table 3 presents results of comparing weights of fresh and stored omega-3 eggs and their main parts. It is obvious that storage period had effect (P<0.05) on albumen weight, meaning that storage duration of eggs affected reduction of albumen weight (from 37.24 g to 36.19 g). Values for weights of eggs, yolk and shell in both fresh and stored omega-3 eggs were similar (P>0.05).

Table 3

Weight of main parts of n-3 PUFA eggs ($\bar{x} \pm s_{\bar{x}}$)

Indicator, g	Fresh (n=30)	Stored* (n=30)
Weight of eggs	60.46±0.35	59.59±0.37
Weight of albumen	37.24±0.28^a	36.19±0.29^b
Weight of yolk	15.53±0.19	15.78±0.14
Weight of shell	7.68±0.07	7.61±0.07

*28 days in refrigerator at + 4°C; $\bar{x}$ = mean value; $s_{\bar{x}}$= error of the mean; numbers in rows marked ^{a,b}differ statistically (P<0.05)

Table 4 overviews results referring to effect of storage period on external and internal quality of omega-3 eggs. Presented results indicate that storage period did not have effect on yolk color. Fresh omega-3 eggs had statistically significantly higher shell

strength and thickness than stored omega-3 eggs (3.312 kg/cm^2 and 0.377 mm, i.e. 2.981 kg/cm^2 and 0.365 mm, respectively).

Table 4

External and internal quality indicators n-3 PUFA eggs ($\bar{x} \pm s_{\bar{x}}$)

Indicator	Fresh (n=30)	Stored* (n=30)
Strength of shell, kg/cm^2	3.312±0.06^a	2.981±0.09^b
Thickness of shell, mm	3.77±0.002^a	0.365±0.002^b
Albumen height, mm	7.09±0.15^a	6.19±0.18^b
Haugh units	83.95±0.93^a	77.97±1.28^b
Yolk color	12.90±0.12	13.10±0.10
pH in albumen	8.90±0.02^b	9.08±0.01^a
pH in yolk	6.00±0.01^b	6.12±0.01^a
AR	0.86±0.35^b	1.86±0.34^a
VN	63.7±0.69^a	60.13±0.50^b

*28 days in refrigerator at +4 °C; $\bar{x}$ = mean value; $s_{\bar{x}}$= error of the mean; numbers in rows marked ^{a,b}differ statistically (P<0.05)

Storage of omega-3 eggs had statistically significant effect also on albumen height, HU and VN, meaning that albumen height was reduced from 7.09 mm to 6.19 mm, HU were lowered from 83.95 to 77.97 and VN was lowered from 63.70 to 60.13. Furthermore, storage period significantly affected (P<0.05) increase of values of albumen pH (from 8.90 to 9.08), yolk pH (from 6.00 to 6.12) and AR (from 0.86 to 1.86). Food has limited shelf life, which primarily depends on food type and storage conditions. Due to poor natural defense barrier, eggs are considered as a foodstuff with limited storage period. According to the Regulations on the quality of eggs (Official Journal No. 115/06 and 76/08), eggs can be placed on market for 28 days under certain storage conditions (in cooling shelves at a temperature up to +5 °C). Freshness of eggs is associated with their quality and affected by storage period (measured in days), as well as by storage conditions (temperature and relative air humidity). Freshness of eggs is counted from the moment of laying until the moment of their use. Every egg producer intends to keep eggs fresh as long as possible, i.e. to preserve indicators of egg freshness for a longer period of time (high values of HU, albumen height, albumen pH, etc.). Intensity of lipid peroxidation in egg yolks is one of the indicators of egg freshness. Higher values of MDA µg/g in samples indicate that oxidation is more intensive and that freshness of eggs decreases. Table 5 shows intensity of lipid peroxidation in egg yolks, both of conventional eggs and omega-3 eggs. It is evident that the storage period of eggs had statistically significant effect on lipid oxidation in egg yolks (P<0.05).

Table 5

Lipid oxidation in yolks of fresh and stored conventional and omega-3 eggs

Indicator	Conventional eggs ($\bar{x} \pm s_{\bar{x}}$)		Omega-3 eggs ($\bar{x} \pm s_{\bar{x}}$)	
	Fresh	Stored*	Fresh	Stored*
µg MDA/g	0.597±0.01^b	0.709±0.02^a	0.510±0.03^b	0.657±0.02^a

*28 days in refrigerator at +4 °C; $\bar{x}$ = mean value; $s_{\bar{x}}$= error of the mean; numbers in rows marked ^{a,b}differ statistically significantly at P<0.05

The value of MDA in conventional eggs fluctuated from 0.597 µg/g for fresh eggs to 0.729 µg/g for eggs stored for 28 days in refrigerator at +4°C. MDA in fresh omega-3 egg yolks was 0.510 µg/g, while in stored eggs it raised to 0.657 µg/g. Strength of brown egg shell was from 3.85 to 4.10 kg/cm^2 (www.isapolutry.com, 2007). Thickness of egg shell varied in dependence on egg weight (*Casiraghi et al.,* 2005). *Şekeroğlu and Altuntaş* (2009) determined statistically significantly ($P<0.05$) thicker shell for medium-weighed eggs (0.400 mm), while the thinnest shell was marked in extra-large eggs (0.382 mm). According to *Kralik et al.* (2006), optimum shell thickness ranges from 0.330 to 0.340 mm. Analysis of our results and comparison with the above mentioned values for shell thickness led to conclusion that values obtained for both examined groups of eggs (conventional and omega-3) in both measurements (fresh and stored eggs) were within the optimum interval, while values referring to shell strength were slightly below recommended values. Conventional eggs exhibited less weight, as well as less shell thickness of eggs stored at +4 °C for 28 days if compared to fresh eggs, which correlates to statements of *Farooq et al.* (2001), *Zita et al.* (2009), *Aygun and Yetisir* (2010), *Moreki et al.* (2011). Omega-3 stored eggs weighed less, but had thicker shell, which was not correlating to results of above mentioned authors. It is assumed that observed difference in shell thickness of stored eggs in comparison to fresh conventional eggs (0.365 mm and 0.377 mm, respectively; $P<0.05$) and of omega-3 eggs (3.160 mm and 3.100 mm, respectively; $P>0.05$) does not correlate with storage period of eggs. It is to conclude that there is a positive correlation between egg weight and shell thickness. Furthermore, it is presumed that values for shell strength, which are slightly lower than optimum, are not resulting from a storage period, but are rather a consequence of other factors, such as age of hens and feeding treatment (*Akyurek and Okur*, 2009). Storage period and temperature are among the most important factors to influence albumen quality and HU values. These values are determined on the basis of total egg weight and height of thick albumen. By storing eggs, structure of albumen is changing and it starts to disperse, causing its height to reduce, and consequently its HU value to decrease. According to specification for the Egg multi tester device, which was applied in measuring HU, values were classified into four freshness categories, where the freshest eggs had HU value above 72 and were marked as AA. If comparing our results with device specification values, it was concluded that investigated eggs (both conventional and omega-3 eggs) were of best freshness and met requirements of the Regulations on quality of eggs. Referring to the area of the Republic of Croatia, measured values for yolk color of table eggs produced by hens in cages range on average from 12.76–13.08 (*Kralik* et al., 2006). As expected, there are statistically significantly lower HU values and albumen height in both stored conventional and omega-3 eggs. This can be explained by the fact that storage of eggs was causing water evaporation and loss of CO_2 from albumen, depending on temperature and relative humidity during storage, due to which stored eggs exhibited lower values of albumen height and Haugh units than fresh eggs. For the same reasons (loss of CO_2), values of albumen and yolk pH were increased during storage of eggs. *Samli et al.* (2005) reported pH values of 7.47 for fresh albumen and 5.75 for fresh yolk, while eggs stored for 2 hours at a temperature of +5 °C exhibited albumen pH of 7.99, and yolk pH of 5.9. These values gradually increase during storage period. Storage period and storage conditions may affect changes in nutritive and sensory quality of food, and lipid oxidation can negatively influence its taste and smell. If a product is enriched with omega-3 fatty acids, lipid oxidation can be more intensive because of a larger portion of unsaturated fatty acids, which are susceptible to oxidation. Determination of *thiobarbituric acid reactive* substances (TBARS) indicates the extent

of oxidation for various fatty acids. Lipid oxidation increases in proportion to increase of MDA concentration in analyzed sample. Our results were in accordance with results published by *Shahryar et al.* (2010), who also pointed out that MDA increased in proportion to egg storage period. They also stated that lipid oxidation was influenced not only by egg storage period, but also by hens' feeding treatment and content of omega-3 PUFA in eggs.

CONCLUSIONS

Research into effect of storage period of eggs (measured on 1st and 28th day) on the quality of conventional and n-3 PUFA eggs resulted in conclusion that storage period of conventional eggs significantly increased ($P<0.05$) albumen pH (from 8.66 to 9.01), yolk pH (from 5.99 to 6.06) and AR (from 0.933 to 1.90), while reducing values for albumen height (from 6.94 mm to 6.16 mm), HU (from 80.80 to 75.06), yolk color (from 8.70 to 7.86) and VN (from 63.26 to 60.50). Analysis of external and internal quality of omega-3 eggs proved that storage period significantly affected ($P<0.05$) increase of albumen pH (from 8.90 to 9.08), yolk pH (from 6.00 to 6.12) and AR (from 0.86 to 1.86), while causing reduction of the following values: shell strength (from 3.312 kg/cm^2 to 2.981 kg/cm^2) and shell thickness (from 0.377 mm to 0.365 mm), albumen height (from 7.09 mm to 6.19 mm), HU (from 83.95 to 77.97) and VN (from 63.7 to 60.13). Both conventional and omega-3 eggs stored for 28 days at +4 °C exhibited statistically significantly more intensive oxidation than fresh eggs of respective groups ($P<0.05$).

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