Italian Journal of Animal Science

Vol.4 - 2005
Supplement 3

Journal and Official Organ
of the Scientific Association of Animal Production (A.S.P.A.)

PROCEEDINGS OF THE 13TH INTERNATIONAL SIMPOSIUM

"ANIMAL SCIENCE DAYS"

HUSBANDRY, FOOD AND ENVIRONMENT. THE NEW CHALLENGES OF THE ANIMAL SCIENCE

AGROPOLIS, PADOVA, SEPTEMBER 12 - 15, 2005

Edizioni
Avenue media
BOLOGNA - ITALY

PROCEEDINGS OF THE 13TH INTERNATIONAL SIMPOSIUM "ANIMAL SCIENCE DAYS" AGROPOLIS, PADOVA, SEPTEMBER 12 - 15, 2005

ORGANISED BY:

Dipartimento di Scienze Animali – Università degli Studi di Padova, Italy

WITH THE PARTNERSHIP OF:

Zootechnical Department, Biotechnical Faculty – University of Ljubljana, Slovenia
Faculty of Agriculture – University of Zagreb, Croatia
Zootechnical Department, Faculty of Agriculture – University J.J. Strossmayer, Osijek, Croatia
Faculty of Animal Science – University of Kaposvar, Hungary

Logistic Organisation: Dipartimento di Scienze Animali
Università degli Studi di Padova, Italy

This Supplement has been published with financial contribution of the Regione Veneto, Italy

The Symposium has been organized with the patronage of
Università degli Studi di Padova
Regione Veneto
Facoltà di Agraria – Università di Padova.

and with the financial support of

Veneto Agricultura

Montorsi S.p.A.

Consorzio Superbrown

Federazione Provinciale Allevatori Trento

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SESSION I

RECENT ADVANCEMENTS IN ANIMAL PRODUCTION AND HEALTH

MAIN PAPER

Breeding for Improvement of Functional Traits in Dairy Cattle

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Paper received April 20, 2005; accepted May 31, 2005

ABSTRACT

Selection programs for increasing milk production per cow have been very successful over time. This success has been partially due to the consideration of few other traits. Unfortunately, many traits related to costs of production and cattle functionality (i.e., "functional traits"), such as fertility and health, are antagonistically correlated with milk yield. Therefore, the average merit for these traits has decreased over time. The decline in functionality, along with increased awareness of the costs of production and animal well-being, has spurred interest in breeding for improvement in functional traits. Unfortunately, factors such as low heritability and lack of data make the selection for functionality more difficult than for production. Research has been able to overcome some of these limitations, at least to some extent, through the development and application of advanced statistical analyses and through indirect selection on genetically correlated traits. Possibilities exist in the future for additional refinement of selection procedures for improvement of functional traits. Computing capacities are continually increasing and more complex but statistically appropriate analysis methods are being developed. Furthermore, genome scans have identified chromosomal regions that have putative associations with functional traits. The bovine genome has been recently sequenced, so the possibility to identify the genes affecting functional traits exists, at least in theory. With low heritabilities and difficulties in measurement, functional traits are the ideal candidates for the application of marker-assisted selection.

Key words: Dairy cattle, Breeding, Functional traits.

RIASSUNTO

SELEZIONE PER IL MIGLIORAMENTO DEI CARATTERI FUNZIONALI NELLA VACCA DA LATTE

I programmi di selezione finalizzati all'aumento della produzione di latte vaccino hanno sempre avuto un grande successo, in parte dovuto al limitato numero di altri caratteri considerati. Sfortunatamente, molti dei caratteri legati al costo di produzione e quelli funzionali, come la fertilità e la salute, sono negativamente correlati con la produzione di latte. Pertanto il loro valore medio è diminuito nel tempo. La riduzione dell'efficienza riproduttiva, insieme all'aumentata attenzione verso i costi di produzione e il benessere animale, ha stimolato l'interesse verso una selezione per i caratteri funzionali. Purtroppo fattori come una bassa ereditabilità e una carenza di dati rendono la selezione per questi caratteri più difficoltosa di quella per la produzione. La ricerca è stata capace di superare alcuni di questi limiti, almeno fino a un certo grado, attraverso lo sviluppo e l'applicazione di analisi statistiche avanzate e attraverso una selezione indiretta sui caratteri geneticamente correlati. In futuro, esistono delle possibilità per un ulteriore perfezionamento delle procedure di selezione finalizzate al miglioramento dei caratteri funzionali. Le capacità di calcolo stanno continuamente aumentando e si stanno sviluppando dei metodi di analisi più complessi, ma statisticamente più appropriati. Inoltre, analisi del genoma hanno identificato regioni cromosomiali che hanno apparenti associazioni con i caratteri funzionali. Il genoma bovino è stato recentemente sequenziato così che la possibilità di identificare i geni che condizionano i caratteri funzionali esiste,

almeno in teoria. Con basse ereditabilità e difficoltà nella misurazione, i caratteri funzionali costituiscono i candidati ideali per l'applicazione della selezione assistita da marcatori.

Parole chiave: *Vacca da latte, Selezione, Caratteri funzionali.*

Introduction

For many years, genetic improvement of dairy cattle was based almost exclusively on increased production per cow. Some selection decisions considered conformation traits, especially in the final choice of dams of sires, but many of the formal indexes produced by national agencies placed most, if not all, of the emphasize on milk production traits. For example, prior to 1994, the official selection indexes published by the United States Department of Agriculture (USDA, 2005) considered only production traits (Van Raden, 2004). The strong emphasis on production was easily justifiable: 1) Milk sales are the primary source of income for most dairy producers; 2) the infrastructure for recording of milk production was available and programs for data collection and storage were active, and 3) genetic improvement is maximized when only a single trait is considered, so selection for functional traits would have decreased the responses obtained for yield traits. Genetic gains in the productive capacity of dairy cattle were remarkable. For example, the yearly genetic trend in the US Holstein population was more than >110 kg per year during the 1990's (USDA, 2005). However, things have changed in the past 10 years. More and more dairy farmers and breeding organizations are becoming concerned not only with how much milk a cow gives, but how she does it. The way a cow functions has gained considerable attention. The term "functional" traits has been coined to refer to the phenotypic characteristics that affect or indicate a cow's well-being. and such traits are now being actively included in selection indexes. The functional traits are a diverse group, including measures of reproductive performance, health and disease resistance, feed consumption, and longevity.

The objective of this manuscript is to discuss breeding for improvement of functional traits, including reasons why they have recently gained in importance, special limitations with functional traits that have affected their rate of adoption, and procedures that have or can be used to allow for their incorporation into breeding programs.

General discussion

Importance of Functional Traits

The reasons for the relatively recent interest in functional traits (and past emphasis on production) are many and diverse. Many of these reasons are indirectly tied to adoption of new technologies and increased production per cow. As production per cow increased, production per farm has increased as well. Thus, a single farmer can now produce a quantity of milk sufficient for many people. The size of farms has increased and the number of farms has decreased. These factors have changed management approaches. In the past, one farmer with a small herd was responsible for all aspects of dairy farming, including milking, feeding, health care, and breeding decisions. In today's large farms, the owner may act primarily as a supervisor and financial manager. In this role, the farmer may be more aware of the costs of production than in the past. The functional traits generally have their impacts on the costs of production, rather than on income. With larger herds, computer software will likely be used for management of financial information, and costs of veterinary intervention, reproductive management and feeding can be organized for simple evaluation. The effects on profit of a particular health problem, mastitis for example, may be more obvious than for a small farm without computerized health records. In large farms, cattle are often housed and managed in groups and the farmer may lack the time or facilities to care for the needs of individual cattle. In small herds, individual cows with functional problems can more easily receive special treatment to allow them to remain productive and profitable. In large herds, such cows may simply be culled, resulting in a loss. In some ways, cattle have become more like machines and trouble-

free cows are important, because individuals with a problem can affect profit by not only directly increasing costs associated with treatment (increased labor, drugs, etc.), but also by disrupting the routine procedures of daily management of rest of the herd.

The increase in production per cow has had other influences on the importance of functional traits, in addition to the indirect effects associated with increased farm size. First of all, breeders may be satisfied with the current genetic trends for production and see improvement of functional traits as a more pressing concern. This is particularly true in countries where the introduction of milk quotas has limited the marginal returns from increasing yield. Furthermore, many health and reproductive traits have negative genetic correlations with production (Pryce *et al.*, 1997). Experiments with lines of cattle selected for increased production have demonstrated that genetically superior cattle for production require more health care and higher treatment costs (Dunklee *et al.*, 1994; Jones *et al.*, 1994). Therefore, the great achievements obtained in genetic improvement for milk yield have been obtained at the cost of diminished fertility, disease resistance and general functionality (Pryce and Veerkamp, 1999). Intense selection for production has had other effects on functional traits in addition to those due to antagonistic genetic correlations between the respective traits. The genetic gains in milk yield were obtained by identifying a few superior animals and using technologies such as artificial insemination and embryo transfer to increase family sizes. This approach to selection has led to a great reduction in the effective population size of most dairy cattle breeds (Weigel, 2001) and increased rates of inbreeding (Kearney *et al.*, 2004). Inbreeding depression is likely to be more severe for functional traits like fertility and survival than for production and has probably contributed to observed phenotypic decreases in these traits.

Producers are more likely to become aware of a health or management problem as the rate of its incidence increases. The threshold of awareness has apparently been crossed by many producers with respect to reproductive efficiency. Phenotypically, measures of fertility have declined markedly in the past 20 years (Beam and Butler, 1999; Van Raden *et al.*, 2004). Many breeders of Holstein cattle in the US have begun experimenting with crossbreeding (McAllister, 2002). It is clear that these breeders currently place a higher value on improved functional traits than on increased production.

Finally, some of the reason for increased awareness of functional traits may be due to the influences from outside forces. The general public is continually becoming more concerned about the source of their food. Many people want to be sure that the animals whose products they are consuming were well treated during the production phase. This concern is due to both a desire to see animals treated well and for concerns about food safety, since healthy animals are likely to produce more healthful food, which is free from pathogens, antibiotics and other drug residues.

Obstacles to Selection for Functional Traits

Breeding for improvement of functional traits presents some difficulties, and this fact may also help to explain why functional traits have only recently gained what seems now to be well-deserved attention. First of all, functional traits generally have much lower heritabilities than do the typical production traits. Table 1 has estimates of heritabilities for production and functional traits from a group of studies in which both types of traits were evaluated together. The estimated heritabilities for the production traits ranged from around 0.20 to nearly 0.50 for the yield traits, averaging around 0.30. The heritabilities reported for fat percentage were generally greater than for yield from the same study (Uribe *et al.*, 1995). In contrast, reported values for the heritabilities of the functional traits were much lower. Among the traits evaluated in these studies, the interval between calving and first estrous or first insemination had the highest heritability estimate (from Royal *et al.* 2002). The reported value was 0.16, which was lower than the lowest reported heritability for the production traits. In addition, other studies found much lower values for the same trait. Heritability estimates for the other traits ranged from 0.01 to 0.15 (Table 1). With heritabil-

Table 1. Heritabilities[1] of production, functional, and indicator traits.

Trait type	Trait	Heritabilty
Production	Milk yield	0.17 to 0.45
	Fat yield	0.25 to 0.47
	Protein yield	0.20 to 0.34
	Fat %	0.31 to 0.41
Functional	Calving interval	0.02 to 0.07
	Interval for return to estrous	0.02 to 0.16
	Number of services	0.02
	Mastitis incidence	0.03 to 0.15
	Cystic ovaries	0.02
	Retained placenta	0.01 to 0.02
	Ketosis	0.08
	Milk fever	0.04 to 0.09
	Longevity	0.04 to 0.10
Indicator	Body condition	0.10 to 0.58
	Change in body condition	0.07 to 0.10
	Somatic cell count	0.14 to 0.20
	Udder depth	0.18 to 0.33
	Teat length	0.21 to 0.33
	Foot angle	0.09 to 0.15
	Lactation persistency	0.18

[1] *From Short and Lawlor (1992); Jairath et al. (1995); Uribe et al., (1995); Van Dorp et al., (1998); Dechow et al., (2001); Royal et al., (2002); Berry et al., (2003); and Muir et al., (2004); Carlén et al. (2005).*

ities so low, selection for genetic improvement for functional traits was generally considered futile, or at least extremely problematic. Reliable sire evaluations for traits with such low heritabilities require much larger daughter groups (>>100 daughters per sire) than those that were typically generated for the precise evaluation of production. Accurate genetic evaluation of cows is essentially impossible for traits with heritabilities so low. Furthermore, low heritability indicates that influences other than genetics play the major role in the phenotypic variability in a trait, so intervention through changes in the environment with management was considered the most logical approach to obtain improvement. In addition to (and possibly at least partially because of) the low heritabilities for the functional traits, another factor limiting genetic selection has been a lack of data. Selection for increased production was possible because milk-recording programs were already established to collect the necessary data. In most countries outside of Scandinavia, little data is routinely collected on health and other functional traits. Data collection is expensive, and the gathering and organizing of data on functional traits specifically for genetic evaluation has always been difficult to justify economically. In fact, genetic evaluation was not the main driving force behind the organization of the current milk recording programs. Such programs were first established for management and culling; genetic evaluation was then recognized as an opportunity to take advantage of and glean more value from the system.

A final shortcoming of functional traits is that many of them have properties that, relative to production, make them more difficult to deal with

from the perspective of data recording and statistical analysis. In some cases, obtaining an objective measurement is difficult, expensive, or almost impossible. For example, mastitis is the most important disease economically for dairy production. Thus, a genetic evaluation based on its incidence could be of great value in a selection program, especially considering its antagonistic genetic relationship with production. In theory, such a program could be based on farmers recording mastitis incidence. However, one problem with this approach may be that different farmers likely have ideas about what constitutes an infection. In Scandinavia, this problem has been avoided somewhat, as the genetic evaluation is based on treatments by veterinarians. However, variability is still likely to exist among veterinarians (Kelton *et al.*, 1998) and, in the end, the farmer makes the decision on whether or not to call the veterinarian. These factors may, in part, be responsible for the low estimates of heritability for mastitis incidence (Table 1). Extending the example, mastitis exists in both clinical and subclinical forms. The selection program in Scandinavia is based on the clinical form, but subclinical mastitis is actually responsible for the majority of economic losses from udder infections (Raubertas and Shook, 1982). Considering this factor, the ideal data collection procedure should probably be based on tests for the presence of bacteria pathogens in the udder, to detect both clinical and subclinical mastitis, but such as system would require enormous costs.

Another advantage that production traits have over many functional traits is that the data for yield are continuous and tend to be normally distributed. Lactational production per cow can take just about any values between 5000 and 25,000 kg, with most of the data centred around a population average. Data with these properties are easy to analyse statistically with a standard linear model. Most of the statistical procedures commonly used for genetic evaluation assume that the dependent variables (or at least the residual effect) are normally distributed. Furthermore, the genetic model generally believed to be correct is an infinitesimal model, which assumes that the genetic effect contributing to an animal's phenotype is the sum of the effects of many genes. This leads to a continuous and normally distributed genetic effect.

In contrast, many functional traits are not normally distributed. Many are dichotomous, taking one of two possible values. For, example, cows can be either sick or healthy, pregnant or open, dead or alive. In other cases, ordered categories have been created, to help assign a numerical phenotype to a trait that is otherwise ambiguous. For instance, a cow may have no problem calving (y = 1), a few problems (y = 2), a lot problems (y = 3), or surgery (y = 4). Even when traits values are continuously distributed, they may not be normally distributed. For instance, the average herd life of a dairy is around 36 months (Short and Lawlor, 1992), but many animals will be culled quite early and thus survive only a month or two after their initial calving. Still others can be quite long lived and surpass 100 months. This results in a skewed distribution. In all of these cases, either a special statistical model should be applied or the data should be transformed to yield a statistically appropriate analysis. In general, the special statistical model requires more complicated software and increased computing time and expense. Transformation can yield estimates that are difficult to interpret on the original phenotypic scale. For these reasons, the standard linear model is often applied for genetic evaluation, regardless of the distribution. Such a procedure results in decreased estimates of heritability and lower potential selection accuracy (Gianola, 1979). Estimates of breeding values (EBV) from the linear model and statistically appropriate model are usually highly correlated (Boettcher *et al.*, 1999), but can still result in significant re-ranking of sires. The most appropriate method is thus recommended, although a simple evaluation is superior to no evaluation. A final shortcoming is that even when traits are continuously distributed and can be relatively easily recorded, such as for the interval from calving to first estrous, official recording would nonetheless be of the "B" type, according to the International Committee for Animal Recording (ICAR, 2005).

Practical Approaches to Selection for Functional Traits

Despite the aforementioned difficulties associated with many functional traits, many countries

have been able to start implementing national genetic evaluations for selected traits and, in some cases, international evaluations (Interbull, 2005). In many cases, the inherent problems with functional traits have been overcome or circumvented by imaginative approaches. The first of these is taking advantage of the opportunities for indirect selection. Indirect selection is based on the recording and genetic evaluation of a trait that is genetically correlated with a trait of interest, rather than the trait itself. This approach is often used when the trait of direct interest has low heritability, is difficult or expensive to measure, or both. Response to indirect selection can be greater than to direct selection if the two traits are highly correlated and if the correlated trait has a markedly higher heritability than the trait of interest (Falconer and Mackay, 1996). A classic example of this approach is the use of somatic cell count (SCC) to select for mastitis. As mentioned previously, collection of data for mastitis incidence is challenging. First, in many countries, mastitis incidence is not routinely recorded. In instances where mastitis data is recorded, the phenotype is usually dichotomous and is based on veterinary treatment, as requested by the farmer. Heritability thus tends to be quite low, usually less than 0.10 (Table 1). However, SCC is often recorded as part of the management data offered by milk recording agencies and large historical databases exist in many countries, as it can have a direct economic value, by influencing the price of milk. The genetic correlation between mastitis incidence and somatic cell count is in the range of 0.60 to 0.80 (Carlén *et al.*, 2002). The phenotype can be recorded objectively and is continuous and normally distributed following a logistic transformation (Ali and Shook, 1980). As a result, estimates of heritability for SCC tend to be 2 to 3 times higher than for mastitis incidence (SCC). In addition, somatic cell score is an indicator of both clinical and subclinical mastitis.

Other possibilities for indirect selection can be identified. Examples of heritabilities for indicator traits are in Table 1, and most tend to be higher than heritabilities for the functional traits. Persistency of lactation has been suggested as a trait to be considered for indirect selection. In theory, cattle that have relatively low peak yield and maintain production at a reasonably high level may be less subject to metabolic problems associated with negative energy balance (Sölkner and Fuchs, 1987). Although this relationship has yet to be fully demonstrated, the possible use of persistency has gained attention because the recent adoption of test day models has opened up the possibility to calculate EBV for persistency. Heritability estimates for persistency can to be at least 5 times greater than estimates for metabolic and fertility traits (Table 1). Body condition score (BCS) has also been proposed as a criterion for indirect selection against metabolic disorders and to help improve the reliability of genetic evaluations for reproductive traits (e.g., Dechow *et al.*, 2001). Estimates of heritability for BCS range from moderate to high, whereas heritability estimates for metabolic disorders and fertility traits tend to be low (Table 1). Although BCS, unlike SCS, is measured subjectively, evaluators can be trained together and a reasonably cohesive system can be developed. In many countries, the scoring of BCS has been assigned to classifiers for type traits. These experts will generally score tens of thousands of cows in a given year. Change in BCS during a lactation may be a more informative trait than average BCS and random regression could be used to develop EBV for specific parts of the lactation where loss of body condition is especially detrimental.

The more traditional type traits can also be used for indirect selection. Feet and leg traits are associated with lameness and other locomotive disorders (Boettcher *et al.*, 1998b). A selection index that considers multiple feet and leg traits can yield higher selection accuracy than direct selection against lameness and some countries currently include such an index in the national breeding goal (e.g. Canadian Dairy Network, 2005). In addition to SCC, udder traits are also genetically correlated with mastitis incidence. In general, genetic correlations between udder traits and mastitis incidence tend to be lower than between mastitis and SCS, but heritability estimates are usually greater (Boettcher *et al.*, 1998a). For maximum accuracy, information on SCS and udder traits can be combined into an index for selection against mastitis (e.g. Boettcher *et al.*,

1998a). Traits related to body dimension can be used to help to select for increased feed efficiency. Large cows need to consume more feed for maintenance than do small cows and this difference can be accounted for in an index to select for total economic merit (e.g. Van Raden, 2002). Increased size also tends to be negatively associated genetically with longevity.

In fact, many type traits have a significant genetic relationship with longevity (Short and Lawlor, 1992). This fact allows for the possibility to create a multi-trait index to select indirectly for increased longevity. In some respects, longevity does not seem to be as obvious a candidate for indirect selection as do certain other functional traits For example, the heritability for longevity is not as low as typically observed for fertility or disease resistance traits. Also, longevity can be measured accurately and objectively, using only the information routinely recorded by milk recording agencies. However, the problem with longevity data lies in the timing of data recording. Cows do not fully express longevity until they leave the herd. Considering the currently high genetic trends for production, many bulls will be removed from active service due to low EBV for milk before they have enough daughters with recorded longevity to obtain an accurate progeny test. Type traits, however, are usually recorded during a cow's first lactation and can thus be used to give an immediate indirect EBV for longevity.

Although longevity has a tangible economic value (unlike some traits used for indirect selection), it can also be considered a trait for indirect selection. Cows can be culled for a wide number of reasons, ranging from high disease incidence to low fertility to behavioural problems. In some respects, longevity can be considered an implicit index for selection for all of these additional functional traits, each weighted by its relative importance as informally assigned by the breeder. In animal breeding circles it has been discussed whether a more detailed index, formally calculating EBV and economic values for the component traits underlying longevity, is even needed, or if longevity is a reasonable and efficient proxy for general health and profitability.

Technologies leading to faster and more efficient computers and new algorithms have allowed for the introduction and adoption of more appropriate statistical methods and software for non-linear estimation of genetic parameters and genetic evaluation of functional traits. A specific example can be cited for longevity. The Survival Kit, a software package developed by Ducrocq and Sölkner (1998), applies survival analysis for evaluation of longevity. Survival analysis analyses the effect of an animal's genetics on the probability that she will be culled at time t, given she has survived up to time t. It has the particularly attractive quality of being able to correctly account for animals that have not yet finished their productive lives at the time of data collection (and thus have not fully expressed their longevity). The records from these animals are considered to be censored on the date of data collection and are used only to evaluate risk of culling at time points up to the time of censoring. Many countries, including most of Europe, have adopted this methodology for their respective national genetic evaluations for longevity (Interbull, 2005). In addition, survival analysis has also been used to analyse other functional traits based on times to a given event, such as fertility (Schneider *et al.*, 2005) and mastitis infection (Carlén *et al.*, 2005).

The relatively recent developments in statistical analysis have also been particularly helpful for the evaluation of functional traits. The introduction to animal breeding applications of Bayesian analysis with Markov Chain Monte Carlo (MCMC) methods (e.g. Wang *et al.*, 1993) and the use of generalized linear mixed models (e.g. Tempelman, 1998) have been especially useful. The former uses iterative procedures to generate samples from the distribution of genetic parameters, conditional on the data available. This process decreases the amount of computer memory required for an analysis, relative to other methods that require maximization of complex likelihood functions. These approaches have been used primarily to analyse traits with categorical distributions, by implementation of the threshold model. The threshold model assumes that the variability underlying a categorical trait is determined by continuous variable called "liability", and there exist thresholds that determine into which category the observed phe-

notype falls (Falconer and Mackay, 1996). The MCMC procedures continually sample liability values, conditional upon the observed categories and other parameters in the model, and these "augmented" data are then analysed with a standard linear model. Threshold models have been applied for the analysis of fertility (Averill *et al.*, 2004), mastitis (Rekaya *et al.*, 2003), longevity (Boettcher *et al.*, 1999), calving ease (Luo *et al.*, 2002), and lameness (Boettcher *et al.*, 1998b), among other functional traits.

Future Possibilities for Functional Trait Selection

The future holds further opportunities to refine selection on functional traits. At the current time, MCMC methods are primarily used for estimation of genetic parameters, but generally not for routine genetic evaluation. Although they demand relatively little memory to implement, MCMC approaches can require significant time, due to the need to generate many samples from the respective posterior distributions. They also would (or could) produce posterior distributions of EBV for all animals, which could involve the need for great deals of data storage, depending on the implementation of the evaluation. However, technological advancements in computing hardware may soon eliminate such obstacles. These advancements my also enable the application of more complex statistical models for the analysis of functional traits. For example, Detilluex and Leroy (2000) noted that SCC data are a combination of data from two distributions, from healthy and infected cattle, and proposed the use of a finite mixture model for genetic analysis. This concept was further developed by Ødegard *et al.* (2003) and Boettcher *et al.* (2005). In these studies, the mixture model was found to be superior to the standard linear model, at least based on statistical measures of fit to the data. More research is still required, however, to refine the model and to understand the results and implications on a biological basis.

Molecular genetics may also provide a useful tool for selection to improve functional traits. According to Meuwissen and Goddard (1996), the traits that stand to reap the most benefits from marker assisted selection are those that have low heritabilities, are difficult or expensive to measure, can measured only after the desired age for selection, or can be measured in only one sex. Most functional traits have at least one of these characteristics. Genome scans have already identified numerous chromosomal regions where possible quantitative trait loci for functional traits seem to be located (e.g. Schrooten *et al.*, 2000). The bovine genome has been recently sequenced and is available in public databases. Different research projects are underway for the discovery of single nucleotide polymorphisms. These tools, along with genetics maps and databases from other species should allow for the eventual identification and characterization of multiple genes that have influences on functional traits.

Conclusions

For many years, the genetics of functional traits of dairy cattle were largely ignored as breeding programs emphasized increasing production per cow. Functional traits were either considered less important or more difficult to evaluate and phenotypic improvement was based on environmental modification rather than breeding. This trend has changed in recent years, as producers have become more concerned about the costs of production and advancements in data recording and statistical analyses have made genetic evaluation of functional traits more feasible. The interest in breeding for functionality is likely to increase in the future as new and existing analysis procedures become routinely adopted and opportunities for marker assisted selection for functional traits become available.

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MAIN PAPER

Automation in dairy cattle milking: experimental results and considerations

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Paper received April 26, 2005; accepted June 7, 2005

ABSTRACT

The results of two experimental programs financed to the Istituto Sperimentale per la Zootecnia are presented. The objective of the two Italian programs was the verify if automatic milking is a suitable practice for Italian dairy system. Results are summarised and compared to those obtained in other international projects. Results refer to animal behaviour, milk yield, milk quality an animal welfare. In a trial comparing cows milked with an automatic milking system and cows milked in a milking parlour, we observed that when the temperature and humidity are very high cows reduce their activity, have lower milking frequency and milk yield than in cold seasons. In comparison to milking parlour, automatic milking system did not increase milk yield which was affected significantly by season, stage of lactation, parity, season per treatment and parity per treatment. The causes of the negative results obtained by this group and by other international groups are discussed. We also presented the results obtained in four trials thereby four appetizers or flavourings were tested to improve efficiency of automatic milking system. Comparing the two milking systems, automatic milking determined a worsening of milk quality, but from these data is not possible to exclude the possibility to use automatic milking for Grana Padano and Parmigiano Reggiano-type cheeses. Animal welfare is not negatively influenced by automatic milking system, which has the potentiality to improve the control and care of cows.

Key words: Dairy cattle, automatic milking.

RIASSUNTO

AUTOMAZIONE DELLA MUNGITURA DELLE BOVINE DA LATTE: RISULTATI SPERIMENTALI E CONSIDERAZIONI

A partire dal 1998 in Europa si sono diffusi i robot di mungitura, che effettuano automaticamente l'attacco dei gruppi di mungitura, eliminando quindi ogni intervento dell'uomo in questa operazione. Nel 2003 vi erano almeno 2200 aziende, soprattutto europee, che utilizzavano almeno un sistema di mungitura automatico. La possibilità di ridurre i costi del lavoro è la principale motivazione che ha spinto gli allevatori ad introdurre nei loro allevamenti un sistema di questo genere, ma ve ne possono essere ancora delle altre: consentire alle vacche di avere un comportamento più naturale, aumentare la produzione di latte, dare all'azienda una immagine positiva, migliorare le condizioni di lavoro, rendere più socialmente accettabile il lavoro dell'allevatore. All'Istituto Sperimentale per la Zootecnia è stato finanziato un progetto dal Ministero delle Politiche agricole e Forestali, per verificare se la mungitura automatica è una pratica idonea al sistema produttivo italiano. La prima fase della ricerca è stata quella di osservare ciò che accadeva in un allevamento sperimentale dopo l'introduzione di un robot di mungitura; la seconda quella di confrontare due gruppi di bovine munte in un sistema automatico e in sala di mungitura tradizionale, al fine di valutare gli effetti sul benessere animale, la produzione e la qualità del latte. Inoltre sono riportati i risultati di un altro esperimento finanziato dalla Regione Lombardia, riguar-

dante l'impiego di appetibilizzanti o aromatizzanti nel mangime distribuito nel robot per migliorarne l'efficienza. I risultati esposti riguardano il comportamento animale, la produzione e la qualità del latte ed il benessere animale. In un confronto tra vacche munte in un sistema automatico con quelli munti in una sala di mungitura, abbiamo osservato che con temperatura ed umidità elevata le bovine riducono la loro attività, la frequenza di mungitura e la produzione di latte si riducono rispetto alle stagioni più fredde. In confronto alla sala di mungitura, la mungitura automatica non determina un aumento della produzione di latte, che è invece influenzata dalla stagione, dallo staio di lattazione, dall'ordine di parto, dall'interazione tra stagione e sistema di mungitura e dall'interazione tra ordine di parto e sistema di mungitura. Nella relazione sono anche discusse le possibili cause dei risultati negativi ottenuti da questo gruppo e da altri gruppi internazionali. Abbiamo inoltre presentati i risultati in quattro prove con le quali abbiamo tentato di migliorare l'efficienza del sistema impiegando sostanze appetibilizzanti o aromatizzanti inserite nel mangime distribuito nell'autoalimentatore del robot. Queste prove non hanno dato risultati positivi sino ad ora. La qualità del latte è stata per alcuni aspetti peggiorata, tuttavia non è possibile escludere la possibilità di impiegare il sistema automatico di mungitura per la produzione di formaggi a lunga stagionatura, anche perché i primi rilievi sulle forme non sono negativi. Non è neppure risultato negativo l'effetto sul benessere degli animali. Al contrario la mungitura automatica possiede una grande potenzialità per migliorare il controllo e la cura degli animali.

Parole chiave: *Bovini da latte, mungitura automatica.*

Introduction

Milking is a labour-expensive activity that requires also skilled personnel. This is the reason why automatic milking of dairy cattle aroused a great interest among breeders in many countries in the world. Automatic milking differs from traditional mechanic milking because cluster attachment is made automatically, so that no operation of the milking process requires the presence of the stockman.

Studies on fully automated milking started in the mid-seventies, but only in 1992 the first commercial dairy farm had a milking robot in The Netherlands. Adoption of this technology went very slowly till 1998 when a significative number of milking robot was sold for the first time. The latest statistics of farms using an automatic milking system (AMS) in the world was presented by de Koning and Rodenburg (2004). In 2003 about 2200 commercial farms had at least one milking robot. Most of them had middle-size herds and 1 to 3 boxes; however there are some examples of large dairy herds adopting an AMS.

Automatic milking requires a big financial investment that is justified in those countries and conditions where labour costs are high. For this reason, AMS are diffused where labour is expensive and the average size of the farms is small or middle. In table 1 there are some data concerning the distribution of automatic milking systems worldwide. Most of them are in Europe (particularly in The Netherland, France, Sweden, Denmark, and Germany) and only a very few are in USA, where the production costs are limited through the high number of cattle per herd.

The possibility to reduce labour costs is not the

Table 1. Diffusion of milking robots (year 2002). From de Koning and Van der Vorst (2002).

Country	AMS (n)	Country	AMS (n)
The Nederlands	520	Finland	29
France	269	Switzerland	25
Sweden	209	Italy	25
Denmark	200	Norway	19
Japan	70	Ireland	15
Canada	55	USA	9
Spain	40	Israel	5
Belgium	36	Austria	5
UK	33	TOTAL	1745

AMS: automatic milking system

only reason that induced the farmers to adopt an AMS. Additional motivations are: more natural behaviour of cows, increased milk yield, a better image of the farm, better labour conditions, a more social life of the farmers etc.

Automatic milking is not simply the adoption of a new type of milking machine, but it is especially a new method to rear cattle, which requires some changes in feeding and housing and which has some relevant consequences on milk yield, milk quality, cows' behaviour and welfare.

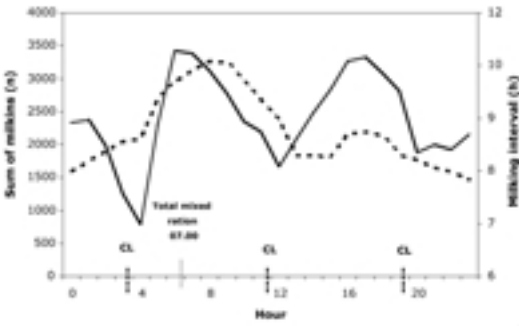
In order to study the effects of the introduction of automatic milking, an important European Program was funded. Some other experimental projects were also carried out thanks to national or regional programs. One of these was financed to the Istituto Sperimentale per la Zootecnia, Cremona, Italy, by the Italian Ministry of Agricultural and Forestry Policies. The first step of this program was to observe what happened in an experimental herd after changing from mechanical to automatic milking. A second step was to compare two groups of cows milked with mechanic or automatic milking machine in order to verify the effects on animal welfare, milk production and milk quality. In addition, two further experiments, funded by Regione Lombardia, were conducted to study milk persistency of cows milked with AMS and improvement of efficiency of AMS through feeding management. The final objective of both programs was the verify if automatic milking is a suitable practice for Italian dairy system. In this paper the results of these programs are presented, compared to those obtained by other European groups, and the future perspective of automatic milking are discussed. The data that are presented have been already published (Abeni *et al.*, 2002; Speroni *et al.*, 2003; Migliorati *et al.*, 2003; Pirlo *et al.*, 2004; Speroni *et al.*, 2004), even thought in a preliminary form in many cases, because most results of the experiments have been just submitted for publication or are still in progress.

General discussion

Animal behaviour

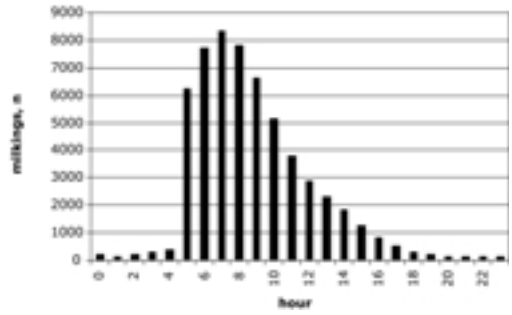
The major difference between automatic and traditional milking is that in the first case cows

Figure 1. Sum of milkings per hour (——) in a period of 22 months and of milking interval (------) during the day.
CL: cleaning of automatic milking system. (Speroni *et al.*, 2003).



are free to go to the milking machine when they want and milkings are distributed throughout the day. In figure 1 the patterns of milkings and of milking intervals we have observed are presented (Speroni *et al.*, 2003). The milkings were performed rather continuously, but not constantly over the day: the drops correspond with cleaning of AMS. The lowest number of milkings was observed early in the morning, the highest was observed just few hours later. This peak is consistent with the feeding schedule of total mixed ration and the lazy cows fetching (between 06.00 and 09.00). The average milking interval is high because most cows do not go to milking during the night, otherwise the second peak in the afternoon corresponds to a short average milking interval because most cows have already been milked in the morning. The overall milking interval was 9 h 23 m 50 s (figure 2); 12.5 % of the milkings occurred after an

Figure 2. Milking intervals.
(Speroni *et al.*, 2003)



interval of 6 h or less and 19 % longer than 12 h; the 4,5 % of all milking intervals were longer than 16 h.

Milk yield

Cows are motivated to go to the AMS because there they are fed some concentrate through an automatic feeder. The consequence is that cows are generally milked more than 2 times a day, with some exceptions (Table 2). Potentially, this could have a great effect on milk yield because it is well known that the increase of milking frequency has a positive effect on milk yield (Smith *et al.*, 2002). Milking frequency depends on several managing and environmental factors such as cow traffic, temperature and humidity, feeding system. We have observed a reduction of presentation to AMS in spring and summer if the Temperature and Humidity Index (Ingraham, 1976), is over 70 for a prolonged period of time (Figure 3).

Figure 3. Pattern of average milk yield per cow with milking parlour (MP) or automatic milking systems (AMS) under different condition of temperature and humidity index (THI). From Pirlo *et al.* (2004).

Percentage of lazy cows (animals that, without any apparent cause, do not go to the AMS) influences milking frequency. This percentage is influenced by herd density, concentrate composition and amount fed in the AMS (Rodenburg and Wheeler, 2002), meaning that several managing aspects should be adjusted in order to stimulate

Table 2. Literature data for milking frequency under automatic milking system.

Author	Average milking frequency	Traffic or condition
de Konning and Ouweltjes, 2000	2.5	
Wendl *et al.*, 2000	2.4	guided
van't Land *et al.*, 2000	2,93	free
	2,93	selectively guided
	2,71	guided
Hopster *et al.*, 2000	3.0	guided
Harms *et al.*, 2002	2,29	free
	2,63	guided
	2,56	selectively guided
Svennersten-Sjaunja *et al.*, 2000	2,38	confined
	1,94	grazing
Spörndly and Wredle, 2002	2.49	grazing
	2,45	
Ketelaar-da Lauwere *et al.*, 2000	2.5-2.9	free with selection in AMS
	3.1-2.8	free with selection before AMS
	2.9-2.8	free with selection before AMS and waiting area
	3.0-3.3	guided with waiting area
Thune *et al.*, 2002	2.56	guided
	2.39	selectively guided
	1.98	free
Abeni *et al.*, 2002	2.85	selectively guided
Speroni *et al.*, 2004	2.67	selectively guided winter and autumn
	2.51	selectively guided summer and spring

Table 3 Effect of different sources of appetizers and flavouring on milk yield (MY), visits to automatic milking system (visits), number of daily milkings (milkings), and milking interval (MI). Difference between treatments were never significant.

	MY (kg/d)	Visits (n)	Milkings (n)	MI (hh.mm.ss)
Experiment 1				
Control	25.6	3.88	2.68	10.03.42
Treatment: combination of aromatic substances	25.7	3.75	2.64	9.30.05
Experiment 2				
Control	24.1	3.58	2.34	10.25.16
Treatment: natural highly intensive sweetener	23.7	3.53	2.35	10.25.28
Experiment 3				
Control	24.1	3.88	2.44	9.59.48
Treatment: aromatic sweetener	23.7	4.43	2.52	10.27.44
Experiment 4				
Control	25.9	4.12	2.51	9.57.34
Treatment: test modifier	25.6	3.93	2.49	11.15.22

the cows to move to AMS. In order to stimulate the voluntary access of the cows into the AMS, we supposed to give concentrates added with highly attracting substances through the feeder in the AMS.

In tables 3 there are the results of four experiments we conducted about the use of flavouring and appetizing substances to attract cows to the AMS. Forty Italian Friesian cows were used to test four concentrates fed in the AMS; the concentrates were added with: 1) in experiment 1 aromatic substances, a gustatory dominance of fenugreek flavour; 2) in experiment 2 a natural highly intensive sweetener; 3) in experiment 3 an aromatic sweetener; 4) in experiment 4 a test modifier, a strong liquor test with low flavour (Migliorati *et al.*, 2003). We did not observe any significant difference and further trials have been done or are in progress to find out effective attracting substances.

In table 4 the results of the experiment thereby we compared a group of cows milked with AMS with another group milked in a milking parlour (Pirlo *et al.*, 2004). Total milk yield of both groups was not affected by milking system, however it was affected significantly by season, stage of lactation, parity season per treatment and parity per treatment (figure 3).

Most of the scientists and companies sustain that automatic milking increases milk yield. This is largely accepted, but the results of the experiments (Table 5) and data of field studies available till now are very inconsistent. In some cases they gave very promising results, with an increase of production ranging around 10% (Kruip *et al.*, 2002; Shoshani and Chaffer, 2002), but the vari-

Table 4 Means and standard deviation of milk yield (Pirlo *et al.*, 2004).

	n	Mean (kg/d)	SD
AMS primiparous	30	27.1	8.83
AMS pluriparous	18	32.5	10.57
AMS Total	48	29.0	9.81
MP primiparous	39	28.3	7.03
MP pluriparous	18	29.4	13.16
MP Total	57	28.7	9.3

AMS: automatic milking system; MP: milking parlour.

ed the data about some characteristics of milk yielded by cows milked with AMS or with milking parlour. We observed a decrease of fat percentage, an increase of SCC and of free fatty acids. Some technological characteristics are also negatively influenced by AMS: natural creaming and titratable acidity which are important parameters in long-ripened cheeses, such Grana Padano or Parmigiano Reggiano. However, the expert cheese maker did not noticed any difference during the whole process. Weight loss during the first 7 months of ripening proceed correctly in both cheeses and no blowing was recorded. In a preceding study (Abeni *et al.*, 2002) we did not observe any negative effects of AMS on technological characteristics of milk, but we obtained an improvement of curd firmness after the introduction of the AMS into the experimental farm in Cremona.

From these data is not possible to state if long-ripened cheeses can be produced with the milk deriving from an AMS, but the results seem promising and studies should be continued not to exclude the possibility to use automatic milking for Grana Padano and Parmigiano Reggiano-type cheeses.

Animal welfare

As for any other technology, automatic milking should respect animal welfare. The European research project had a special section about animal welfare and our project also took in consideration this issue.

Cows in an AMS are expected to visit the robot voluntary. However, in a herd there are both high- and low-ranked cow, and lay-out and management should be in such a way to permit to give access to milking and feeding to both ranks. A second concern refers to the reactions of cows which are not allowed to pass gates. A third concern is the adaptation of cows passing from the traditional milking to AMS. A review on welfare of cows milked with AMS was made by Wiktorsson and Sørensen (2004).

In our experiment (Pirlo *et al*. 2004), we evaluated the capacity of heifers to cope the starting of lactation in an AMS and in a conventional system. We observed a slight difference in stress measure. Starting after calving, the cortisol values were generally higher in AMS than conventional milking (figure 4). In AMS group there were more cows with spike values of cortisol in blood, suggesting a cronic stress situation with a stronger responsiveness to any stress-like stimulus, such as bleeding. Nevertheless, we can that the effect we measured was negligible compared to the advantages for the animal and the farmers; in addition, there is a large agreement that AMS is largely comparable with traditional milking for the animal welfare (Wiktorsonn and Sørensen, 2004).

Table 6. Least square means and standard error of milk parameters of primiparous cows in MP and AMS (Pirlo *et al.*, 2004).

		MP	AMS	P
Fat	%	3.61±0.07	3.33±0.09	0.02
Protein	"	3.54±0.04	3.50±0.03	NS
Lactose	"	5.17±0.,03	5.17±0.02	NS
SCC	ln (10^3/ml)	4.37±0.15	4.81±0.11	0.02
Urea	mg/100 ml	24.69±0.46	25.12±0.34	NS
Freezing point	°C	-0.5293±0.0006	-0.5291±0.0004	NS
FFA	meq/100 g of fat	0.531±0.039	0.700±0.030	0.001
Natural creaming	% of fat	39.74±1.50	36.11±1.24	0.06
Median fat globule Ø	µm	4.55±0.11	4.61±0.08	NS
pH		6.706±0.010	6.745±0.007	0.002
Titratable acidity	°SH/100 ml	7.143±0.100	6.759±0.072	0.002
Casein N	% of N	75.73±0.39	75.62±0.25	NS
NPN	% of N	5.49±0.13	5.72±0.09	NS

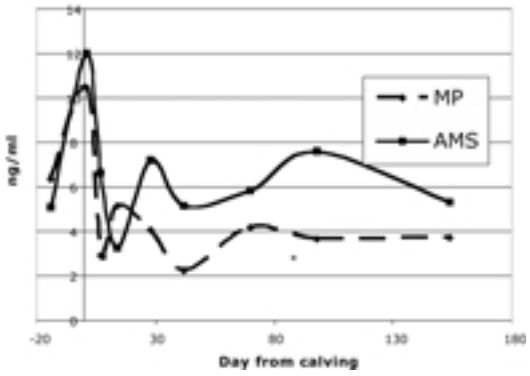
AMS: automatic milking system; MP: milking parlour.

Animal characteristics and selection

Percentage of cows that should be culled changing from traditional to automatic milking is one of the main concerns of farmers, although it does not appear so relevant from an economical point of view. It seems also that this problem is less important than what appeared when the first milking robots were introduced into commercial herds.

We do not know studies about the percentage of cows which were culled because they were not suitable for such a milking; recently we proposed a form where the most relevant characters are evaluated. All the characters have a lower and a higher limit; but only some characters have limits that must be respected (Migliorati, 2003). The form can be used by the farmer before deciding to buy a milking robot and can be also used for the selection of cows in the perspective of a larger diffusion of milking robots.

Figure 4. Plasma cortisol in primiparous cows milked with AMS or milking parlour (MP)



Conclusions

Automatic milking is already a reliable technology which seems to be well accepted by farmer and consumer. A great EU project and many other national projects, as that we made in Italy, let us say that the automatic milking represents an opportunity to improve animal welfare and milk quality. Even though there are some negative results for milk quality, robot milks cows in a precise and regular way and this very promising for an improvement of milk quality. Even for welfare the results are very promising, because the difference in behavioural and physiological analyses observed between AMS and milking parlour are very little; but, above all, AMS gives the opportunity of a precise and continuous control of cows. The data given by the AMS are precious for early detection of several disorders and can put in evidence anomalies in behaviour and production. There are still some concerns about milk yield whose increase is lower than expected. For a further increase of milk yield, strategies to have more regular movement of cows to the AMS are needed.

Perspectives

Automation is still at the beginning in animal husbandry. In the future, the man will have the opportunity to change his work from a manual activity to control operations. Electronic will help him to care animals individually and precisely also if they are in large herds. Milking robot is the focal point of this process, may be because it does the most complicated operation. In the future, further electronic devices and biosensors will be added to it and cattle will be kept according their individual needs.

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SHORT COMMUNICATION

Comparison of statistical models to estimate daily milk yield in single milking testing schemes

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ABSTRACT

Different statistical models were compared to estimate daily milk yield from morning or evening milking test results. The experiment was conducted on 14 family farms with 325 recorded cows. The amount of explained variance was higher for models including the effects of partial milk yield, the interval between successive milking, the interaction between partial milk yield and the milking interval and the farm (R^2 = 0.976 for AM, R^2 = 0.956 for PM) than for models including partial milk yield effect only (R^2 = 0.957 for AM, R^2 = 0.937 for PM). Estimates of daily milk yield from linear models were more accurate than those obtained by doubling single milking weights. The results show that more complex model gives the best fit to the data. Differences between models according to determination and correlation coefficient were minor. Further investigations on larger sets of data are needed to draw more general conclusion.

Key words: Milk recording, AT method, Daily milk yield, Estimation.

Introduction

The accuracy of daily milk yield estimation is an important factor for single milking testing schemes. In order to estimate daily milk yield from single milking weights various models have been proposed. Depending on the model, different factors that affect milk production were take into account, like parity, stage of lactation and the interval between successive milkings (Hargrove, 1994; Cassandro *et al.*, 1995; Klopčič, 2004). The milking interval is the most important factor when estimating daily milk yield from partial yields. The objectives of this study were to develop and compare models to estimate daily (24 hours) milk yield from single morning (AM) and evening (PM) milking records.

Material and methods

The data used in this study were 2,994 individual test-day milk yield records collected from

Table 1. Descriptive statistics of milk traits

Trait	Mean	SD	CV	Min	Max
Daily milk yield (kg)	19.78	6.99	35.35	4.00	59.90
Morning milk yield (kg)	11.10	3.93	35.42	2.00	32.70
Evening milk yield (kg)	8.67	3.25	37.45	1.90	27.20
Nightly interval (hour)	13.63	0.74	5.40	11.47	15.68
Daily interval (hour)	10.48	0.74	7.04	8.33	13.43

Table 2. Selected statistical models for estimation of daily milk yield.

Model	df	Factors included in model			
		m	t	m*t	F
A	2	YES	NO	NO	NO
B	3	YES	YES	NO	NO
C	4	YES	YES	YES	NO
D	17	YES	YES	YES	YES
E[1]		m*2			

[1] *doubling single am or pm milk yield*

*df = degree of freedom, m = AM or PM milk yield, t = daily or nightly milking interval in minutes, m*t = interaction between m and t, F = farm*

november 2004 to march 2005 on 325 cows reared in 14 family farms in Croatia. At each recording, milk yield was measured in the evening and in the morning. Three samples were taken from each cow: one sample at each milking (evening and morning) and one proportional milk sample that was taken for regular recording. Variability of daily, morning and evening milk yield, as well as day and night interval between successive milkings are reported in table 1.

For statistical analysis the SAS/STAT package was used (SAS Institute Inc., 2000). Daily milk yield was estimated by four different statistical models and by doubling AM or PM.

The models differed by the number of the effects (Table 2). Models were compared on the basis of the determination coefficient (R^2), variability coefficient for standard error (CV_e) and root mean square error (σ_e).

Results and conclusions

Determination coefficient (R^2) values for models based on morning milk yield ranged from 0.957 in model A, which included only partial milk yield as covariate, and 0.976 in model D which included effects due to the farm, milking interval, partial milk yield as well as the interaction between partial milk yield and the milking interval (Table 3).

R^2 values for models based on evening milkings ranged from 0.937 (model A) to 0.956 (model D). These results indicate that estimation of daily milk based on morning milking will be more reliable than on evening milking. Reliability is slightly higher for more complex models than in the simplest one (model A).

Correlation between actual and milk yield estimated on AM milking varied from 0.978 (model A and E) to 0.988 (model D) and from 0.968 (model A

Table 3. Determination coefficient (R^2), variability coefficient for standard error (CV_e) and root mean square error (σ_e) for models used to estimate daily milk yield from single milking weights.

Model	df	Morning milking			Evening milking		
		R^2	CV_e	σ_e	R^2	CV_e	σ_e
A	2	0.957	7.354	1.454	0.937	8.906	1.761
B	3	0.971	6.055	1.197	0.953	7.709	1.524
C	4	0.973	5.872	1.161	0.953	7.688	1.520
D	17	0.976	5.561	1.099	0.956	7.318	1.447

Table 4. Parameter estimates for models A and B ($P < 0.01$).

Model	Parameter	Morning milking		Evening milking	
		estimate	SE	estimate	SE
A	intercept	0.4726	0.1429	1.7109	0.1648
	partial yield	1.7385	0.0121	2.0830	0.0178
B	intercept	15.7233	0.7351	14.0955	0.7165
	partial yield	1.7437	0.0099	2.1662	0.0161
	interval	-0.0187	0.0009	-0.0209	0.0012

and E) to 0.979 (model D) on PM milking. These results are in agreement with those reported by Liu *et al.* (2000). It is obvious that high correlation indicates the best suitability of the model.

Estimation of daily milk yield can be based on the regression coefficients given in table 4. All parameter estimates were statistically significant. The reason for choosing only A and B models lay in the fact that this models are simple to use in practice and they still give adequate accuracy in estimation of daily milk yield. We recommend use of model B in practice, but there is a need for further investigations with larger sets of data.

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SHORT COMMUNICATION

Genetic Relationship Between Body Condition Score, Fertility, Type and Production Traits in Brown Swiss Dairy Cows

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ABSTRACT

This study aimed to investigate the relationship between body condition score (BCS), calving interval (CI), angularity (ANG), strength (FV) and milk yield (MY) on Brown Swiss cattle using data collected in the alpine provinces of Bolzano-Bozen and Trentino. The data set consisted of 28,538 test day records of BCS and MY from 3,282 Brown Swiss cows in lactation reared in 109 herds; production traits were merged with 13,796 repeated individual calving interval records and 38,711 type traits records. A multi-traits REML animal model was used to estimate (co)variance components, with repeated observations. Heritability estimates for BCS, FV and ANG was 18%, 18% and 27%, respectively, while estimates for CI was very low (2%). Genetic correlations between CI and BCS was -0.44; between BCS and ANG was -0.64; between BCS and MY was -0.35; between ANG and CI was 0.12. In conclusion, the selection for MY and ANG negatively affect fertility and average condition score of Brown cows. BCS recorded during lactation could be proposed as a useful trait for indirect selection aimed to improve fertility of cows.

Key words: Body condition score, Calving interval, Milk yield, Type traits.

Introduction

Genetic selection on production traits is reducing reproductive efficiency of dairy cattle (Castillo-Juarez *et al.*, 2000), increasing susceptibility to some diseases and the risk of culling (Dematawewa and Berger, 1998). Hence, functional traits, and possibly also fertility traits, should be included as part of the breeding goal, but the possibilities of actually using reproductive information as a selection tool for breeders are limited. Calving interval (CI) is traditionally the main measure of reproduction during the productive life of the animal, particularly in dairy cattle (Rege and Famula, 1993) and it is more likely to be recorded accurately when compared to other fertility traits.. However, CI might not be the most desirable measure of fertility to be included in a breeding goal, because CI data are available on fertile animals only, and not on culled cows. Moreover, CI is only available for multiparous cows, and so represents an inefficient trait for breeding companies, whose decisions on bulls are based on information available earlier in their bull's daughters. Thus, traits possibly correlated with CI but available earlier than CI, such as BCS and some type traits, may be useful in selection of dairy cows.

Aim of this study was to estimate genetic parameters for BCS, CI, some type traits and milk yield using data recorded on Brown Swiss cows herded in the Bolzano-Bozen and Trentino provinces.

Material and methods

Data set consisted on test day records of production and functional traits collected by Superbrown consortium from September 2001 to

Table 1. Descriptive statistics of milk yield, BCS, calving interval (CI) and type scores.

	N. of records	Mean	S.D.	Minimum	Maximum
BCS, score	28,538	3.21	0.46	1.50	4.75
CI, d	13,716	408	73	211	750
Milk yield, kg/d	28,538	26.5	7.6	5.2	61.3
Angularity, score	38,711	26.4	7.3	1	50
Strength, score	38,711	25.9	8.5	1	50

September 2004. BCS was collected monthly on all lactating cows. Type traits (angularity – ANG and strength – FV) were available only in primiparous cows and were extracted by data base of National Breeders Association of Brown Swiss (ANARB). The final data set consisted of 28,538 test day records of BCS and MY from 3,282 lactating Brown Swiss cows herded in 109 farms and of 13,796 individual CI records and 38,711 individual type traits. Genetic parameters were estimated using REML. Variance component estimation (VCE) package (Groeneveld, 1996) was used to estimate (co)variance components according to the following models:

$$BCS_{ijklmn} = \mu + HTD_i + age_j + cdib_k + clat_l + a_m + c_m + e_{ijklmn}$$

$$MY_{ijkmn} = \mu + HTD_i + age_j + cdib_k + a_m + c_m + e_{ijkmn}$$

$$CI_{ijklmn} = \mu + HYS_i + age_j + prod_k + dur_l + a_m + c_m + e_{ijklmn}$$

$$T_{ijkmn} = \mu + HYS_i + age_j + ISPET_k + a_m + e_{ijkmn}$$

where T = ANG or FV; HTD_i = fixed effect of herd-test-day; age_j = fixed effect of age at calving within parity; $cdib_k$ = fixed effect of days in lactation; $clat_l$ = fixed effect of milk production within parity; HYS_i = herd year season of calving; prod= fixed effect of milk yield within parity; dur_l = fixed effect of length of lactation; $ISPET_k$ = fixed effect of inspector; a_m = random animal effect; c_m = permanent environmental random animal effect; and e_{ijklmn} = random residual effect. Variance and covariance components were estimated for MY, BCS, CI, ANG and FV. A pedigree data consisted on 92,436 animals. All animals had sire information and the large majority of cows had dam identifications as well. Pedigrees for as many of the dams and sires as possible were also included in the pedigree file.

Results and discussion

Descriptive statistics of traits are shown in Table 1. Average test day milk yield exceeded 26 kg/d. Average CI was close to 410 days, suggesting a decline of fertility in Brown Swiss cattle. Average BCS during lactation was equal to 3.21, ranged between 1.50 and 4.75 and exhibited a variation coefficient greater than 14%.

Table 2. Additive genetic variance ($\sigma^2 g$), heritability (h^2), standard error (s.e.) of h^2, and genetic correlation for traits of concern (standard error of genetic correlation ranged from 0.075 to 0.187).

	$\sigma^2 g$	h^2	s.e. of h^2	BCS	CI	ANG
BCS	0.028	0.178	0.018			
Calving Interval (CI), d	70.220	0.017	0.006	-0.438		
Milk yield, kg/d	2.901	0.105	0.013	-0.345	0.152	
Angularity (ANG)	12.889	0.270	0.011	-0.643	0.123	
Strength	9.203	0.184	0.010	0.302	-0.236	0.250

Additive genetic variance, heritability and genetic correlations for traits of concern are shown in Table 2. BCS showed a moderate h^2 estimates (18%), although h^2 estimated in this study was lower than that found for BCS in Holstein cows by Gallo *et al.* (2001). As expected, CI showed a very low h^2 value (2%), and this is in agreement with results of previous studies on Holsteins (Pryce *et al.*, 2001). Also type traits showed moderate h^2 estimates, ranging between 18 and 27 % for ANG and FV, respectively.

Genetic correlations between traits are shown in Table 2. BCS was negatively correlated with CI, MY and ANG but positively correlated with FV. Milk yield appeared positively correlated with CI.

Therefore, a decrease of average condition score and an increase of CI are to be expected as indirect responses of selection for increase milk yield.

Also ANG was positively correlated with CI; therefore, more angular cows have longer CI. Conversely, BCS could be useful for predict an early and reliable fertility genetic index of bulls for their daughters' fertility because it is available since the first days of lactation.

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SHORT COMMUNICATION

Evaluation of nutritional status of dairy cows based on milk analysis results

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ABSTRACT

Milk composition data, milk fat percentage (g/100ml), milk protein percentage (g/100g) and milk urea content (mg urea/dl) of 50,000 individual milk samples of cows under milk recording were used to evaluate nutritional status of dairy cows on Croatian farms. Analysis of average values of milk components indicates that most of the animals (60%) had a ration well balanced in energy and fibre, but ration protein deficiency could be a major characteristic of nutrition of considerable number (61.5%) of cows. Using milk analysis results it is possible to estimate major problems in dairy cow nutrition, predict and prevent possible metabolic and reproductive disturbances.

Key words: Nutritional status, Milk fat, Milk protein, Milk urea.

Introduction

Milk composition depends greatly on ration composition – changes in concentration and ratio of fibre and energy in ration affect milk fat percentage and protein percentage (Tamminga, 2001) and milk fat to milk protein ratio, thus making this milk components a possible indicator of ration composition. Also, milk urea content could be used as an indicator of dietary crude protein concentration (Broderick and Clayton, 1997) and energy to crude protein ratio (Godden *et al.*, 2001). In Croatia there are 77,000 cows under milk recording, and since 2002 Central laboratory for milk control besides milk fat, protein content and somatic cell count offers milk urea analysis and provides dairy farmers with necessary milk composition data to monitor nutritional status of their herds. Dairy cow nutrition in Croatia has specific characteristics caused by the farm structure (herd size), breed structure, level of knowledge in nutrition, use of traditional feeds and other factors. Based on milk composition analysis this paper attempts to point out possible errors in nutrition.

Material and methods

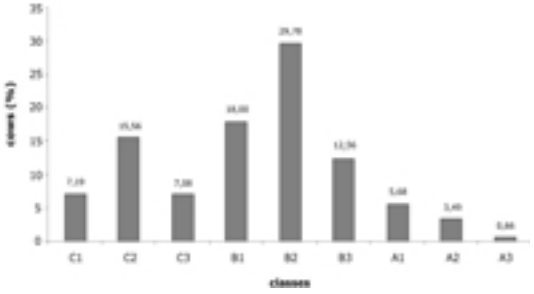
Milk composition data, for 50,000 random individual milk samples of entire population of cows in the state, were collected during year 2004. Average size of farms is 9 cows per herd, population consists of 78% Simmental, 19% Holstein and 3% of other breeds. Milk fat and protein content was determined by infrared spectrophotometry method and milk urea content by enzymatic method. All samples were preserved with azidiol. Classes of cows according to milk protein content and milk fat/protein ratio and according to milk protein and urea content are reported in table 1 and 2, respectively.

For statistical analysis the SAS/STAT package was used (SAS Institute Inc., 2000).

Table 1. Classes of cows according to milk protein content and milk fat/protein ratio.

Classes	Protein (%)	Fat/protein ratio
C1	≤ 3,20	≤ 1,10
C2	3,21 – 3,80	≤ 1,10
C3	≥ 3,81	≤ 1,10
B1	≤ 3,20	1,11 - 1,50
B2	3,21 – 3,80	1,11 - 1,50
B3	≥ 3,81	1,11 - 1,50
A1	≤ 3,20	≥ 1,51
A2	3,21 – 3,80	≥ 1,51
A3	≥ 3,81	≥ 1,51

Figure 1. Percentage of cows per classes according to milk protein content and milk fat/protein ratio



Results and conclusions

Results of the analysis indicate that 60.34% of animals (classes B1, B2, B3, figure and table 1) had a milk fat/protein ratio 1.1-1.5, which could be taken as optimal (Babnik *et al.*, 2004), 9.83% (classes A1, A2, A3, figure and table 1) had milk fat/protein ratio greater than 1.51 which could indicate energy deficiency and fibre surplus in ration, and 29.83% (classes C1, C2, C3, figure and table 1) had milk fat/protein ratio less than 1.1 indicating fibre deficiency and energy surplus. According to milk fat/protein ratio and milk protein content 29.78% of animals (class B2 figure and table 1) could have a balanced content of energy and protein in ration.

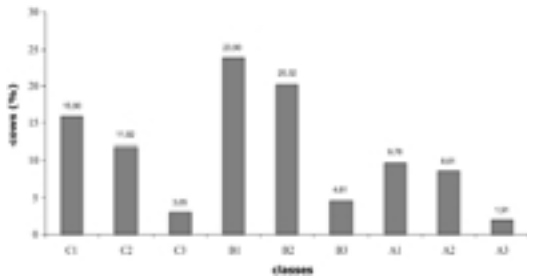
Based on milk urea content as a single parameter protein content in ration could be deficient for 49.58% of animals (C1, B1, A1 figure and table 2, less than 15 mg urea/dl), in excess for 9.57% of animals (A3, B3, C3 figure and table 2, more than 30 mg urea/dl/) and adequate for 40.85% of animals (A2, B2, C2 figure and table 2, 15-30 mg urea/dl). If milk urea content and milk protein content are both taken as parameters about 20.32% of animals (B2 figure and table 2) could have ration adequate in energy and proteins, 6.52% excess of proteins in ration (A3, B3 figure and table 2) and as much as 61.5% (A1, B1, C1, C2 figure and table 2) could be suffering from protein deficiency.

These milk composition data are in agreement with our expectations and current nutritional practices on dairy farms. Generally farmers themselves produce energy rich feeds (corn silage,

Table 2. Classes of cows according to milk protein and urea content.

Classes	Protein (%)	Urea (mg/dl)
C1	≤ 3,20	≤ 15,00
C2	≤ 3,20	15,01 – 30,00
C3	≤ 3,20	≥ 30,01
B1	3,21 – 3,80	≤ 15,00
B2	3,21 – 3,80	15,01 – 30,00
B3	3,21 – 3,80	≥ 30,01
A1	≥ 3,81	≤ 15,00
A2	≥ 3,81	15,01 – 30,00
A3	≥ 3,81	≥ 30,01

Figure 2. Percentage of cows per classes according to milk protein and urea content.



wheat) on their own land which makes them easily available. Protein feeds have to be purchased on relatively high prices which causes their low content in ration and thus protein underfeeding of most animals. Result of these nutritional practices can be observed on milk control results.

Analysis of nutritional status of dairy herds in Croatia based on milk composition data can suggest that considerable number of cows (49.58-61.5%) has a protein deficient rations, and 60% of animals has ration balanced in fibre and energy. Based on milk composition analysis ration quality and balance can be estimated and corrected and milk quality improved. Taking into consideration that this method could be a good diagnostic tool when properly used, and that it is easily available to dairy farmers and causes small extra costs, it is expected that it will be more widely applied than it is at the present.

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SHORT COMMUNICATION

Fattening and slaughter traits of four rabbit genotypes

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ABSTRACT

Growth rate of 4 genotypes, two lines of Slovene SIKA meat rabbit (maternal line A and sire line C, both 50 rabbits), hybrid AxC (50 rabbits) and crossbred AxCal (A and Californian, 38 rabbits) was recorded from weaning (35 days of age) to slaughter (93 days). Growth performance and slaughter traits of AxC were different from line A (live weight at 91st day: A 2720, C 3158, AxC 3043 g (P£0.05), heterosis effect (H) +3.38%; carcass weight: A 1490, C 1787, AxC 1716 g (P£0.05), H +5.65%; dressing percentage: A 54.1, C 55.6, AxC 55.5% (P£0.05); H +1.26%. Weaning weight of AxCal was significantly lower than of AxC (AxCal 843, AxC 1050 g, P£0.05), but at the end of the experiment the weight of AxCal rabbits was very close to AxC (AxCal 2958, AxC 3043 g, P>0.05). Other growth and slaughter traits did not differ between AxC and AxCal group.

Key words: Rabbits, Genotypes, Growth, Slaughter traits.

Introduction

In Slovenia, a selection line SIKA for meat production was formed to meet Slovenian needs for breeding rabbits. In conditions of insufficiently developed rabbit market the two-way crossing was established. In Rabbit centre of Biotechnical Faculty, the selection of maternal SIKA line A started in 1988 and the sire SIKA line C in 1995. The genetic factors have high effect on growth rate, proportions of separate digestive organs and carcass quality. Selection on high growth rate can change fattening and slaughter traits (Piles *et al.*, 2000). The production traits of pure SIKA lines are continually recorded from the beginning. The aim of present study was to find out the growth and slaughter traits of hybrid SIKA rabbits (two-way cross between maternal A and sire C line), compared with crossbred between maternal A and Californian rabbits.

Material and methods

Growth rate of 4 different genotypes was recorded from weaning (35 days of age) to slaughter (93±1 days). Genotypes were: two lines of Slovene SIKA meat rabbit (maternal line A and sire line C, both 50 rabbits), hybrid AxC (linecrossing between A and C, 50 rabbits) and crossbred AxCal (between maternal SIKA line A and Californian rabbit, 38 rabbits). Animals were fed a commercial pelleted diet (17% CP, 14% CF) *ad libitum*; feed intake and weight of rabbits were recorded weekly. Average feed intake, daily weight gain (DWG) and feed conversion ratio (FCR) were calculated in each week and during the entire trial. Slaughter weight, warm carcass weight (without head and distal parts of legs, including liver and kidneys), weight of liver, kidneys and separate digestive organs (with their contents) were measured at slaughter (17 rabbits of line A,

Table 1. Live weight of rabbits of different genotypes.

	A		C		AxC		AxCal	
Age (days)	LSM	± SE	LSM	± SE	LSM	± SE	LSM	± SE
36	827 [a]	11.92	1050 [b]	12.17	974 [c]	11.92	843 [a]	13.69
42	1057 [a]	21.65	1284 [b]	22.18	1194 [c]	22.73	1079 [a]	24.37
49	1245 [a]	43.31	1508 [b]	41.71	1371 [c]	43.91	1291 [ac]	51.02
56	1330 [a]	39.1	1591 [b]	37.02	1445 [c]	39.71	1379 [ac]	44.32
63	1758 [a]	39.8	2065 [b]	38.23	1926 [c]	41.02	1852 [ac]	45.12
70	2063 [a]	36.71	2384 [b]	35.26	2281 [c]	38.44	2189 [a]	41.62
77	2305 [a]	35.93	2671 [b]	35.03	2577 [b]	38.25	2460 [c]	40.74
84	2521 [a]	38.72	2925 [b]	37.75	2826 [b]	41.22	2720 [c]	43.91
91	2720 [a]	41.09	3158 [b]	40.06	3043 [c]	43.75	2958 [c]	46.59

A - SIKA maternal line; C - SIKA sire line; AxC - hybrids between A and C; AxCal - crossbreds between A and Californian breed;
[a,b] =P≤0.05

14 of C, 15 hybrids AxC and 27 crossbreds AxCal) and the proportions with respect to the slaughter weight were calculated. The heterosis percent for AxC hybrids was estimated by comparison of mean value of hybrids and the mean value of both purebreds: H=((mean AxC-mean A and C)/mean A and C) x 100. Statistical analysis was performed by SAS statistical package (SAS, 1999) with GLM procedure. Effects of genotype and sex as fixed effects and their interaction were included in the model.

Results and conclusions

During the entire trial, the mortality was very high (average 32.4%) with no significant differences between genotypes. The majority of rabbits died in first 3 weeks of experiment (till 56 days of age). Growth performance and slaughter traits (Table 1 and 2) of AxC hybrids were similar to sire line C and both groups had better results than line A. Most estimates of heterosis (Table 3) indicated that linecrossing between SIKA lines is associated with positive effect on growth performance, except for FCR. Similarly it can be concluded for slaughter traits, especially for carcass weight and dressing percentage.

Weaning weight of crossbred rabbits AxCal was significantly lower than that of AxC hybrids (and equal to the weight of maternal line A), but at the end of the experiment the weight of AxCal rabbits was very close to AxC. Rabbits in AxCal group had lighter carcass and heavier stomach than AxC hybrids, while other growth and slaughter traits did not differ between AxC and AxCal groups.

The live weight of AxC hybrids was higher than Italian hybrids (Kermauner and Žgur, 2002) and crossbreds between Pannon White and Danish White rabbits (Szendro *et al.*, 1998), but lower than meat line (Hy+) selected for high growth rate (Dalle Zotte and Ouhayoun, 1998). Crossbreds AxCal were heavier than crossbreds between New Zealand White and Californian (Nofal *et al.*, 1995) and comparable with crossbreds between Pannon White and Danish White rabbits (Szendro *et al.*, 1998).

Because of different dissection procedures it is difficult to compare dressing percentage with other authors: in our experiment it was higher than some results with line C (Kermauner and Žgur, 2002), higher or comparable with maternal lines (Pla *et al.*, 1998) and lower than in lines, selected for high growth (Piles *et al.*, 2000). Proportion of gastro-intestinal tract was lower than in synthetic meat line (Piles *et al.*, 2000) and SIKA sire line C (Kermauner and Žgur, 2002).

Results obtained for Slovene SIKA meat line show moderate heterosis effect in growth performance, but for better expressed linecrossing effect

Table 2. Growth performance during fattening and slaughter traits of rabbits of different genotypes.

	A		C		AxC		AxCal	
	LSM	± SE	LSM	± SE	LSM	± SE	LSM	± SE
DWG (g/d)	33.5 [a]	1.67	39.4 [b]	1.01	38.1 [b]	1.17	40.9 [b]	1.88
Feed intake (g/d)	126 [a]	3.19	137 [b]	2.77	141 [b]	3.21	135 [ab]	5.15
FCR	3.78 [a]	0.07	3.54 [b]	0.06	3.72 [ab]	0.07	3.31 [b]	0.12
Slaughter weight (SW) (g)	2754 [a]	59.82	3215 [b]	65.8	3094 [c]	63.71	3001 [c]	50.26
Warm carcass weight (g)	1490 [a]	36.37	1787 [b]	40.01	1716 [b]	38.74	1651 [c]	30.55
Dressing percentage (%)	54.11 [a]	0.36	55.56 [b]	0.39	55.46 [b]	0.38	54.87 [ab]	0.30
Liver (% SW)	3.23 [a]	0.13	3.19 [a]	0.14	3.64 [b]	0.14	3.59 [b]	0.11
Kidneys (% SW)	0.63 [a]	0.01	0.54 [b]	0.01	0.58 [c]	0.01	0.58 [c]	0.01
Gastro-intestinal tract (% SW)	15.97 [ab]	0.72	14.32 [a]	0.79	15.55 [ab]	0.77	16.87 [b]	0.61
Stomach (% SW)	4.86 [a]	0.21	4.31 [b]	0.23	4.61 [ab]	0.22	5.22 [c]	0.17
Small intestine (% SW)	3.08 [ab]	0.11	2.85 [b]	0.12	3.31 [a]	0.12	3.24 [a]	0.09
Caecum (% SW)	5.31 [ab]	0.21	4.94 [a]	0.23	5.39 [ab]	0.22	5.48 [b]	0.17
Colon (% SW)	2.70 [a]	0.63	2.20 [a]	0.70	2.23 [a]	0.67	2.92 [a]	0.53

A - SIKA maternal line; C - SIKA sire line; AxC - hybrids between A and C; AxCal - crossbreds between A and Californian breed;
[a,b] =P≤0.05

the time from the beginning of the separate selection of maternal and sire lines is probably too short. Namely, the selection of sire SIKA line C started from one part of maternal SIKA line population in the year 1995. In selection of SIKA meat line special attention has to be paid on improvement of FCR.

Table 3: The heterosis estimates (in %) for fattening and slaughter traits of hybrids AxC.

	Heterosis (%)
Weaning weight (at 36 day)	4.06
Live weight at 91st day of age	3.38
Feed intake	6.97
DWG	3.08
FCR	2.68
Slaughter weight (SW)	4.45
Carcass weight	5.65
Dressing percentage	1.26
Gastro-intestinal tract (% SW)	2.13

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SHORT COMMUNICATION

Fattening and slaughter traits of four rabbit genotypes

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ABSTRACT

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Key words: Rabbits, Genotypes, Growth, Slaughter traits.

Introduction

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Growth rate of 4 different genotypes was recorded from weaning (35 days of age) to slaughter (93±1 days). Genotypes were: two lines of Slovene SIKA meat rabbit (maternal line A and sire line C, both 50 rabbits), hybrid AxC (linecrossing between A and C, 50 rabbits) and crossbred AxCal (between maternal SIKA line A and Californian rabbit, 38 rabbits). Animals were fed a commercial pelleted diet (17% CP, 14% CF) *ad libitum*; feed intake and weight of rabbits were recorded weekly. Average feed intake, daily weight gain (DWG) and feed conversion ratio (FCR) were calculated in each week and during the entire trial. Slaughter weight, warm carcass weight (without head and distal parts of legs, including liver and kidneys), weight of liver, kidneys and separate digestive organs (with their contents) were measured at slaughter (17 rabbits of line A,

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Results obtained for Slovene SIKA meat line show moderate heterosis effect in growth performance, but for better expressed linecrossing effect

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FCR	3.78 [a]	0.07	3.54 [b]	0.06	3.72 [ab]	0.07	3.31 [b]	0.12
Slaughter weight (SW) (g)	2754 [a]	59.82	3215 [b]	65.8	3094 [c]	63.71	3001 [c]	50.26
Warm carcass weight (g)	1490 [a]	36.37	1787 [b]	40.01	1716 [b]	38.74	1651 [c]	30.55
Dressing percentage (%)	54.11 [a]	0.36	55.56 [b]	0.39	55.46 [b]	0.38	54.87 [ab]	0.30
Liver (% SW)	3.23 [a]	0.13	3.19 [a]	0.14	3.64 [b]	0.14	3.59 [b]	0.11
Kidneys (% SW)	0.63 [a]	0.01	0.54 [b]	0.01	0.58 [c]	0.01	0.58 [c]	0.01
Gastro-intestinal tract (% SW)	15.97 [ab]	0.72	14.32 [a]	0.79	15.55 [ab]	0.77	16.87 [b]	0.61
Stomach (% SW)	4.86 [a]	0.21	4.31 [b]	0.23	4.61 [ab]	0.22	5.22 [c]	0.17
Small intestine (% SW)	3.08 [ab]	0.11	2.85 [b]	0.12	3.31 [a]	0.12	3.24 [a]	0.09
Caecum (% SW)	5.31 [ab]	0.21	4.94 [a]	0.23	5.39 [ab]	0.22	5.48 [b]	0.17
Colon (% SW)	2.70 [a]	0.63	2.20 [a]	0.70	2.23 [a]	0.67	2.92 [a]	0.53

A - SIKA maternal line; C - SIKA sire line; AxC - hybrids between A and C; AxCal - crossbreds between A and Californian breed;
[a,b] =P≤0.05

the time from the beginning of the separate selection of maternal and sire lines is probably too short. Namely, the selection of sire SIKA line C started from one part of maternal SIKA line population in the year 1995. In selection of SIKA meat line special attention has to be paid on improvement of FCR.

Table 3: The heterosis estimates (in %) for fattening and slaughter traits of hybrids AxC.

	Heterosis (%)
Weaning weight (at 36 day)	4.06
Live weight at 91st day of age	3.38
Feed intake	6.97
DWG	3.08
FCR	2.68
Slaughter weight (SW)	4.45
Carcass weight	5.65
Dressing percentage	1.26
Gastro-intestinal tract (% SW)	2.13

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SHORT COMMUNICATION

Effect of maternal lysine supplementation on the performance of growing rabbits. Preliminary results

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ABSTRACT

The experiment studied the effect of dietary lysine supplementation to rabbit does on the performance and on meat's protein and lysine content of their offspring. Half of the does (n=43) fed control diet (C; Lys: 0.68%), while the other half a lysine supplemented diet (L; Lys: 0.80%) from 3 days before AI until weaning. After kindling, half of the litters of C does were put under C does, while the other half under L does. The same procedure was followed for offspring of L does. After weaning, rabbits fed the same diet (0.68% Lys). Does' dietary treatment significantly affected the weaning weight, however, only lysine supplementation during suckling age had negative effect (340 *vs* 315g for C and L does, respectively; P<0.01). The kit's milk intake, measured at 3rd and 7th day of age, nursed by L does was significantly lower. Other productive and carcass traits did not differ significantly.

Key words: Rabbits, Lysine, Production, Meat.

Introduction

Researchers have paid attention to the threonine, methionine and lysine requirements of does and growing rabbits as well as on the effects on their performance (Colin and Allain, 1978; Maertens and De Groote, 1988; Taboada *et al.*, 1994, 1996; De Blas *et al.*, 1996). Meat production is determined by the muscle development, which occurs at foetal and early postnatal ages (Ouhayoun and Dalle Zotte, 1993). Thus, increasing lysine supplementation at foetal and suckling ages could affect the carcass traits. The aim of the experiment was to study the effect of early lysine supplementation on the productive and carcass traits as well as on the meat quality of growing rabbits.

Material and methods

At the rabbit farm of the Kaposvár University multiparous does were divided into two groups 3 days before AI. One group (n=43) was fed control (C), while the other one (n=43) consumed lysine supplemented (L) diet (Table 1). Two days before AI previous litters were weaned, thus, none of the does were nursing during pregnancy. At the 31st day of pregnancy (30 C and 40 L does became pregnant) parturition was induced by oxitocyne. All kits were removed from the nests and new litters were formed equalized to 9 kits within doe's group (C or L) according to the kits weight. Then, half of the new litters of C does were put under C (CC), while the other half under L does (CL). The same procedure was followed for the litters of L does (LL and LC). Controlled lactation was per-

Table 1. Chemical composition and nutritive value of the diets.

Chemical composition	Diet of does		Fattening diet
	Control (C)	Lysine supplemented (L)	
Crude protein, %	18.1	18.1	16.0
Ether extract, %	4.1	4.1	3.0
Crude fibre, %	14.5	14.5	16.0
Lysine, %	0.68	0.80	0.68
Methionine + Cystine, %	0.64	0.63	0.53
DE MJ/kg	10.68	10.67	10.60

Chemical composition was analytically determined. The crude protein content was determined by Kjeldhal method, the crude fat content by Soxhlet extraction after hydrochloric acid digestion. Amino acid content was determined by gas-chromatography (MacKenzie, 1987).

formed. At the 17th day nestboxes were opened and all rabbits consumed the same fattening diet (Table 1). At the 3rd, 7th and 14th days after parturition milk production was measured by weighing the litters before and after nursing (Table 2). At 21st day of age kits (n=541) were weighed and weaned. The kits' mortality is shown in Table 2. The lightest and the heaviest rabbits were excluded, thus rabbits near to the average body weight in each group were used afterwards (n=343). From weaning to slaughter rabbits were fed fattening diet *ad libitum* (Table 1). Body weight and feed intake were measured weekly. Rabbits were slaughtered at 11 wk of age.

Slaughtering and dissection procedure were done according to Blasco and Ouhayoun (1996). Meat samples of hindleg meat were collected from 15 average weight rabbits per group for meat quality analysis, performed at the Department of Animal Science of Padova. Protein content of hindleg meat was calculated, while its lysine content within protein was analytically determined

Table 2. Effect of maternal lysine supplementation on the performance of offspring.

Traits	Lys during pregnancy (P)		Lys during lactation (L)		SE	Significance (P<)		
	C	L	C	L		P	L	P*L
Number of nursing does			23	36	-		-	
Milk consumption at 3rd day, g/kit			11.2	9.3	0.42		0.021	
Milk consumption at 7th day, g/kit			21.2	18.9	0.51		0.026	
Milk consumption at 14th day, g/kit			26.8	25.8	0.72		0.507	
Kits' mortality, %	12.4	8.54	8.33	11.4	2.55	0.111	0.304	-
Number of rabbits	171	172	167	176	-	-	-	-
Body weight at 3 weeks of age, g	328	326	340	315	2.68	0.973	0.001	0.291
Body weight at 11 weeks of age, g	2560	2534	2571	2525	12.1	0.362	0.072	0.581
Weight gain between 3-11w, g/day	39.8	39.3	39.8	39.4	0.20	0.259	0.290	0.773
Feed intake between 3-11w, g/day	115	115	116	115	1.32	0.976	0.744	0.522
Feed conversion between 3-11w, g/day	2.89	2.93	2.89	2.93	0.03	0.442	0.519	0.999
Dressing out percentage, %	*60.4*	*60.8*	60.6	60.6	0.12	*0.092*	0.995	0.151

C, L: see Table 1

(MacKenzie, 1987). Data were evaluated by two-way ANOVA using the SPSS 10.0 programme package (SPSS for Windows, 1999). Mortality was evaluated by chí²-test. The contrasts "lysine during pregnancy" and "lysine during lactation" were tested.

Results and conclusions

Dietary treatment of does significantly affected the weaning weight, however, it seems only lysine supplementation during suckling age had negative effect (P=0.001; Table 2). This is proved by the milk intake of kits which was lower in rabbits nursed by L does. However, significant difference was found only at the 3rd and the 7th day of lactation (Table 2). Taboada *et al.* (1994) fed diets of different lysine supplementation (0.64→0.82%) to rabbit does. They found increasing weaning weight with increasing dietary lysine until 0.76%. No difference was found in kits' mortality (Table 2).

No significant difference was found in the body weight at 11 wk of age (Table 2), thus rabbits nursed by L does could compensate during the fattening period. The weight gain was similar, and no significant differences were found in feed intake and feed conversion. Lysine supplementation during foetal age had positive influence on dressing out percentage (P=0.092; Table 2), while lysine supplementation during suckling age had no any effect on this trait. Protein content of hindleg meat was somewhat higher in rabbits born or nursed by L does (P<0.10; Figure 1) with a slight improvement on lysine concentration of hindleg meat (ns; Figure 2). The lysine content found in the present work is considerably higher than that reported in literature (6.5-7.0% protein; Moughan *et al.*, 1988). Recent data report values close to those found in the present work (8.7% protein; INRAN, 2000). These discrepancy could derive from the sample (whole body tissue or edible parts for the literature cited and hindleg meat for the present work) or from the different analytical methodology

In conclusion, it was shown that supplementing the maternal diet with lysine during suckling age (from 0.68 to 0.80%) decreased the kits' weaning weight. Lysine supplementation during foetal or during suckling age had no effect on growth performance. However, maternal lysine supplementation could have a positive effect on some meat quality traits of the offspring, correlated to the protein content. To prove the positive effect of dietary lysine supplementation at early stage of body development further study have to be carried out.

Research supported by OTKA TS 044743 and by Hungarian – Italian Intergovernmental S & T Cooperation Programme.

Figure 1. Effect of maternal lysine supplementation on the protein content of hindleg meat

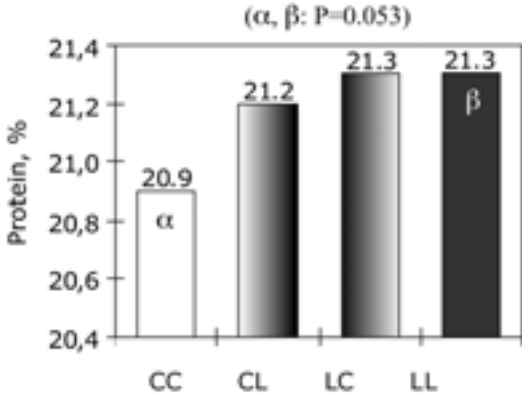
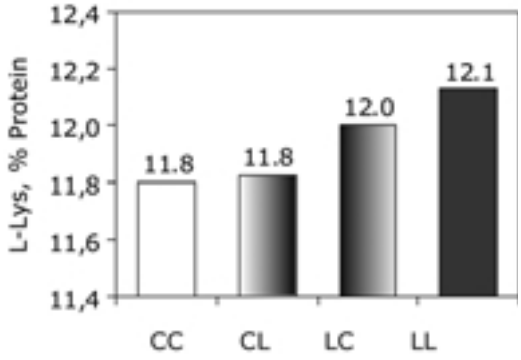


Figure 2. Effect of maternal lysine supplementation on the lysine content within protein



CC: born from C doe and nursed by C doe; CL: born from C doe and nursed by L doe; LC: born from L doe and nursed by C doe; LL: born from L doe and nursed by L doe

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SHORT COMMUNICATION

Genetic parameters of racing merit of Thoroughbred horses in steeplechase races

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ABSTRACT

The aim of this study was to estimate variance components of racing ability in Thoroughbreds involved in steeplechase races. Race results were collected from steeplechase races in France (n=9041), in the United Kingdom and Ireland (n=8314) and contained the results of overall 106 020 runs from 1998 to 2003. Performance was measured by two criteria: earnings and ranks after mathematical transformation. The effects of year, sex, age, and race were considered as fixed, animal, permanent environment and maternal as random. Maternal environmental component for ranks were 0.021 in France and 0.000 in the United Kingdom and Ireland. Estimated heritabilities for the ranking criteria were 0.18 (repeatability 0.33) in France and 0.06 (repeatability 0.19) in the United Kingdom and Ireland. The high genetic correlation between the two traits (0.94 and 0.97) gives the opportunity to find out the most suitable criteria for breeding value estimation.

Key words: Thoroughbred, Steeplechase, Jumping ability, Genetic parameters.

Introduction

The selection of Thoroughbreds is based on their racing performance usually on flat races. Breeders prefer to breed Thoroughbreds for flat races rather than for steeplechase because the horses take part in races from the age of two, and they can have earnings at an early age. In Europe only France, the United Kingdom and Ireland have numerous steeplechase races with a lot of participants. The race track is composed of wooden base and birch or spruce interior obstacles. Plain fences vary in height from a minimum of 135 cm and some are open-ditch fences. Considering the above mentioned statements, the steeplechase race records seems to be the best system to select Thoroughbreds for jumping ability, however the 95% of the participants are geldings.

Most of the authors measure performances for racehorses by earnings and ranks. However these researches based on the datasets of flat race records only. In most cases a mathematical transformation of records is needed (Langlois, 1975).

Earning and mathematical transformations of earnings (log of earning per start, log of annual earnings) have been discussed in several studies (Hintz, 1980; Langlois, 1980; Langlois and Blouin, 2004). In the case of jump races Langlois *et al.* (1996) found heritability near 0.25 (logarithms of yearly earnings) in horses that have taken part in steeple and hurdle races in France from 1950 to 1990.

Ranks also can be a measurement of performance (Langlois, 1980; Langlois *et al.*, 1996). Williamson and Beilharz (1996 and 1998) used position rates in Australia, while Sobczynska and Lukaszewicz (2003) used square root of the finishing position for Arab horses in Poland. Heritabilities were 0.18 (repeatability 0.34) for the

square root of ranks in Poland, and 0.57-0.6 and 0.73-0.74 for performance rates of sires and dams respectievly in Australia. However compared with other studies these latter h^2 values resulted extremely high.

Breeding value estimation is the best way to improve the choice of the parents for the next generation. The aim of this study was to estimate variance components for the two above mentioned traits and find out the most suitable criteria for breeding value estimation.

Material and methods

Race records from the United Kingdom and Ireland have been collected from the Raceform Interactive /commercially available dataset/ and from France-Galop for the French races. Data from 1st of January 1998 to 31st of December 2003 were analysed. Ranks and logarithmic transformations of earnings were used during the analysis to measure performance. "Computed earnings" were calculated for horses without earnings with the method developed by Chico (1994) using SAS 8.2 softwre (SAS, 1999). Computed earnings were equal to half the earnings of the horse that ranked before. Normal distribution of the earnings is obtained by applying a logarithmic transformation (Langlois, 1975). Transformation for ranks was necessary in order to use normalized measure of performance. We used probit function to calculate the expectation of rank k out of n horses in the race. An animal model similar to that developed by Tavernier (1989 and 1990) was used to estimate variance components and breeding values for earnings and ranks using VCE (Groeneveld, 1998) and PEST (Groeneveld, 1990) software. Independent fixed effects were year /from 1998 to 2003/, sex (mare or gelding/stallion), age (3 to 5 in France, 4 to 5 in the United Kingdom and Ireland and 6, 7, 8, 9, 10, 11, 12 and up) and race (6,632 in French and 5,605 in the United Kingdom and Ireland). In France there were 9,041 participants, progeny of 986 sires and 5,927 dams. In the United Kingdom and in Ireland there were 8,314 horses from 1,466 sires and 6,934 dams.

Precorrection for the race effect was needed because the high number of races. Adopting the method by Belhajyahia *et al.* (2003) the performances s_{ij} of horse i in race j was considered to be influenced by two effects, the fixed race effect (r_j) and the random horse effect (H_i):

$$s_{ij} = H_i + r_j + e_{Ij}$$

where e_{Ij} is a random residual, H_i considered as random and r_j is fixed effect.

The final analytical model for the two datasets was:

$$y = Xb+Yg+Yp+Zm+e$$

where y = vector of observations (log of earnings or precorrected transformed ranks = s_{ij}-r_j); b = vector of fixed effects, as year, age, sex; g = vector of genetic values; p = vector of permanent environment to an animal; m = vector of maternal effect (common environment shared to the offspring of the same mare); e = vector of errors while X, Y, Z were the incidence matrices. For earning race effect is not considered because the donation is the manner to evaluate the level of the race. For the ranking value, because mean of s_{ij} in a race is zero it is necessary and it was precorrected.

Results and conclusions

Results of heritabilities for log of earnings and ranks are lower than published before, but these results are not comparable with other studies based on the race resuts of flat races. Considering this heritability, the repeatability and the number of races per horse and year, they can be compared with the values found by Langlois *et al.* (1996). It was 0.25 for heritability and 0.05 for maternal variace component in French steeplechase races for earnings. The maternal environmental component was evaluated at 0.009 (±0.005) for log of earnings and 0.024 (±0.007) for ranks on the French dataset. The same component was lower in the United Kingdom and Ireland, 0.006 (±0.007) for log of earnings and 0.001 (±0.002) for ranks. Genetic correlations between ranks and log of earnings were 0.935 (±0.008) in France and 0.968 (±0.016) in the United Kingdom and Ireland.

Comparing the two data files, lower values were estimated for both the measured traits in the case of the United Kingdom and Ireland. The estimated maternal environment components were different between datasets with lower values (0.006 and 0.001) in the United Kingdom and

Ireland than in France. However, Langlois and Chico (1989) showed a clear difference between the paternal and maternal paths of heredity in racehorses.

Earnings and ranking values in France and in the United Kingdom and Ireland are useful criteria for selection of Thoroughbreds in steeplechase races. Horses take part in these races have also jumping ability, not only speed and stamina. The high genetic correlation between the two traits (0.94 and 0.97) gives the opportunity to find out the most suitable criteria for breeding value estimation. The ranking value which is a normally distributed by construction has a great advantage for comparison between countries because it is the same across countries. This is not to the case of earnings which depends on national policies of donation.

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SESSION II

SAFETY AND QUALITY OF FOOD OF ANIMAL ORIGIN

MAIN PAPER

The future trends for research on quality and safety of animal products

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Paper received May 13, 2005; accepted July 1, 2005

ABSTRACT

Quality must now be considered as a convergence between consumers' wishes and needs and the intrinsic and extrinsic quality attributes of food products. The increasing number of quality attributes which must be considered, increasing globalisation and the heterogeneity in consumption habits between countries are making this convergence progressively more difficult. In parallel, science is rapidly evolving (with the advent of genomics for instance), and a growing number of applications is thus expected for the improvement of food safety and quality. Among the meat and fish quality attributes, colour is very important because it determines, at least in part, consumer choice. The key targets to ensure a satisfactory colour are animal nutrition and management for fish, processing and product conditioning for meat. Tenderness and flavour continue to be important issues for the consumer because eating remains a pleasure. They both determine quality experience which itself influences repetitive purchase. Meat tenderness is a very complex problem which can be solved only by a holistic approach involving all the factors from conception, animal breeding and production, muscle biology and slaughter practice to carcass processing and meat preparation at the consumer end. Today, safety and healthiness are among the most important issues. Unfortunately, animal products can potentially be a source of biological and chemical contamination for consumers. The introduction of both control strategies along the food chain and the development of a food safety management system, from primary production to the domestic environment, are key issues that must be achieved. Despite a high dietary supply of saturated fats by dairy and meat products, it is imperative that professionals involved in animal research and in the associated industry convey the positive nutritional contributions of animal products to both consumers and health professionals. The latter include protein of high biological value, iron, zinc, healthy fatty acids including omega-3 polyunsaturated fatty acids (PUFA) and conjugated linoleic acid. Fish products are well known for their high content in PUFA. Meanwhile, new strategies in terms of antioxidant supply are required to limit PUFA and cholesterol oxidation, which has many detrimental effects including contributing to reduced sensory attributes. Traceability is another quality attribute used to implement food safety and quality. Traceability of an animal's breed and identity, of geographical origin and of diet fed are increasingly important issues demanded by consumers. Safety and quality are thus important issues with increasing complexity, research has to adapt itself to meet this challenge.

Key words: Quality, Safety, Meat, Fish, Dairy products.

RIASSUNTO

LE TENDENZE FUTURE DELLA RICERCA SU QUALITÀ E SICUREZZA DEGLI ALIMENTI DI ORIGINE ANIMALE

Oggi la qualità deve essere considerata come una convergenza tra i desideri e le necessità dei consumatori e i requisiti qualitativi intrinseci ed estrinseci dei prodotti alimentari. Tale convergenza è resa sempre più difficile a causa del numero crescente di requisiti qualitativi che deve essere considerato, della globalizzazione e dell'eterogeneità delle abitudini di consumo tra i vari Paesi. Parallelamente, la scienza si sta evolvendo rapidamente (grazie all'avvento della genomica, ad esempio) e si prevede quindi un numero crescente di applicazioni per garantire maggiore sicurezza e qualità degli alimenti. Tra i requisiti qualitativi della carne e del pesce il colore riveste grande importanza, poiché condiziona, almeno in parte, la scelta del consumatore. Le soluzioni principali per garantire un colore soddisfacente risiedono nell'alimentazione e nella gestione degli animali per quanto riguarda il pesce e nella preparazione e conservazione del prodotto per quanto concerne la carne. La tenerezza e il sapore continuano a rappresentare fattori importanti per il consumatore perché mangiare rimane pur sempre un piacere. Entrambi questi requisiti condizionano l'esperienza di qualità del consumatore e ne influenzano la tendenza a continuare ad acquistare un determinato prodotto. La tenerezza della carne rappresenta un problema estremamente complesso che può essere risolto soltanto adottando un approccio olistico che comprenda tutti i fattori che vanno dal concepimento alla riproduzione e allevamento degli animali, alla biologia del muscolo, alla tecnica di macellazione e al trattamento delle carcasse fino ad arrivare alla preparazione della carne da parte del consumatore finale. Oggigiorno la sicurezza e la salubrità rientrano tra i fattori più importanti in assoluto. Sfortunatamente, i prodotti di origine animale possono rappresentare una fonte potenziale di contaminazione biologica e chimica per i consumatori. L'introduzione di strategie di controllo lungo tutta la filiera alimentare e lo sviluppo di un sistema di gestione della sicurezza alimentare, dalla produzione primaria fino all'ambiente domestico rappresentano obiettivi fondamentali da raggiungere. Nonostante un elevato apporto di grassi saturi nella dieta quotidiana provenienti da prodotti caseari e dalla carne, è essenziale che i professionisti impegnati nella ricerca animale e attivi nell'industria del settore rendano noto sia ai consumatori che agli operatori sanitari il contributo nutrizionale positivo apportato dagli alimenti di origine animale. Questi infatti contengono proteine di elevato valore biologico, ferro, zinco, acidi grassi essenziali, compresi gli acidi grassi polinsaturi omega 3 (PUFA) e gli isomeri dell'acido linoleico coniugato (CLA). I prodotti a base di pesce sono già ben noti per il loro elevato contenuto di PUFA. Nuove strategie sono però necessarie per aumentare l'apporto di antiossidanti per limitare l'ossidazione dei PUFA e del colesterolo, capace di sortire effetti deleteri, compresa la perdita delle caratteristiche organolettiche. La tracciabilità è un altro requisito qualitativo utilizzato per attuare la politica di sicurezza e qualità degli alimenti. La tracciabilità della razza e dell'identità di un animale, dell'origine geografica e della sua alimentazione in allevamento sta diventando un requisito sempre più richiesto dai consumatori. Sicurezza e qualità sono pertanto fattori importanti caratterizzati da complessità sempre maggiore e la ricerca deve adattarsi per fare fronte a questa nuova sfida.

Parole chiave: *Qualià, Sicurezza, Carne, Pesce, Prodotti caseari.*

Introduction

At the beginning of human history, products of animal origin (harvested by hunting, fishing and milking) were considered as noble food contributing to growth, strength, health and longevity. As agriculture developed, the major objective was to satisfy the food needs of humans quantitatively. This objective was achieved in developed countries, and especially in the European Union, which is self-sufficient for most animal products. The development throughout the human history of the market economy, trades and exchanges within and between countries and continents has also permitted some countries to buy sufficient animal products from others to satisfy the quantitative demand of their own populations. Meanwhile, a reduction in the amount of ruminant animal products consumed is recommended due to their high saturated lipid content which is deleterious for human health (Lucas *et al.*, 2005; Valsta *et al.*, 2005). Over the last 30 years, consumption has fallen naturally, though in more recent years any reductions are perhaps temporary in response to some safety crises such as BSE, dioxin issues, foot and mouth disease, etc.

So, while animal production was originally focused on quantity, it has now shifted towards delivering on quality which is high and consistent and improved safety. These are important issues in the European food industry. Quality used to be considered in relation to physical product quality (sensory, nutritional and technological traits, etc). They remain important issues which will be addressed in this paper, but quality also includes

aspects related to production characteristics (e.g. environmental impact, wholesome) and economic performance of the overall food chain (e.g. minimal costs, service quality, etc).

It is also important to emphasise that not only consumers, but also international and national legislation place demands on quality and safety (probably due to the perceived demand of consumers by the political organisations). Last but not least, the increasing demand for quality and safety traits has induced a proportional increase in traceability which is considered to ensure quality and safety at the consumer end. Although this is not immediately apparent, traceability is now included in quality attributes.

Since the concept of quality is becoming more sophisticated, the first part of this paper will attempt to define this concept and to explain why the demand of quality and safety for food products of animal origin is increasing in the European and the World markets. This has also been discussed recently (Hocquette and Gigli, 2005). The following sections will present the major issues for important basic quality traits, namely (i) sensorial quality (colour, texture and tenderness, flavour and juiciness), (ii) nutritional value, (iii) safety and (iv) traceability. The more important food products of animal origin will be considered (dairy products, meat products and fish) although most of the given examples will be for meat products.

1. Quality and safety concepts

Why the European characteristics of animal production systems has/will influence(d) quality and safety concepts

The livestock and fishery systems in Europe have several characteristics which have modified and will further modify, the concepts of quality and safety of animal products. These characteristics mainly relate to the high degree of heterogeneity across the European Union and to difficulties of being included in the global world market. They are sometimes specific for either the meat or the dairy products sectors.

Heterogeneity appears firstly in the structure of agricultural holdings: 60% use less than 5 hectares in Greece, Italy and Portugal compared to 68 hectares on average in the UK ; 53% are localised in less favoured areas or mountainous regions, some are mixed holdings (mixed cropping and/or mixed livestock) or specialised holdings with wide variations between European countries. In the case of meat, some countries are more than self-sufficient (350% for Ireland and Denmark) and others in deficit (54% for Greece). Heterogeneity also lies in the different European cultures: Mediterranean countries favour lamb; Scandinavian countries traditionally consume a lot of milk, while consumption of fish and aquatic products has steadily increased over the past decade in all the European countries (FAO data). Milk consumption has however decreased in Scandinavian countries probably due to the link between heart disease and milk consumption in humans. In the case of cattle, some countries (such as France, the UK and Ireland) are strongly oriented towards beef meat production and others (Denmark, Germany, Italy and The Netherlands) towards milk production. Ewe milk is mainly produced in Mediterranean countries, and goat milk mainly in France. This type of production is however important for rural life, especially in mountainous regions, dry areas and some southern European countries. In the case of pig production, contrasting trends have been observed in the different European countries: an increase in Spain despite limitation in feed resources, an increase in Denmark but a decrease in Germany despite concerns about environment issues in both countries, a decrease in the UK due to animal welfare concern and the foot and mouth disease crisis (Aumaître and Rosati, 2004). Fish is supplied both by fisheries and by fish production. Products from fisheries have remained stable since the seventies while fish production is increasing (28 % of world consumption, 13 % in Europe) to meet the consumer demand.

The difficulties for the European food economy to be included more in the world market are linked to several factors. They include increasing labour costs and a decrease in labour forces on the farms, a strong deficit in the protein feed supply for animal nutrition, and some specific European legislations concerning animal welfare, environment and safety issues. Among the latter, strong limitations

on the use of feed additives (antibiotics, and any other growth promoters) have been recommended in the European Union in contrast to other countries. Some recommendations have also been given for the rearing conditions of laying hens. The reduction of protein level in the diets of monogastrics is also one of the most efficient solutions in the prevention of soil pollution by excess nitrogen and phosphorus (Aumaître and Rosati, 2004). But the world market is important for the European economy because the EU is, for instance, among the largest exporters of pork and of dairy products. More generally, international trade issues are continually increasing. This is globalisation, which has brought with it, as side effects, a reduction in the international prices of agricultural products. Opening up international markets and obvious price differentials in different markets have a huge impact on quality attributes of products from animal origin. One major factor is that lifestyle and consumer expectations of food are tending to converge throughout the world. Consequently, players have more and more common tools and criteria to assess the quality and safety of animal products (Aumaître, 1999), despite cultural, political and economical differences between countries across the world, and even across the European Union as discussed above. Divergences in legislation between countries, occurring concomitantly with developments in international markets, increase the complexity of the problem, as well as the continuous appearance of new products and the evolution of the food science throughout the world. Large differences in food preferences and lifestyle exist between countries. In Europe, for example, these differences in food preferences are important as it is the basis of cultural differences which most importantly helps to maintain the mosaic of farming and food production practices.

The definition of safety and quality

Many experts have attempted to define safety and quality. Safety is often included in the list of quality attributes and refers to the absence of adverse health effects due to the presence of biological and chemical contaminants in food products. Most available definitions of quality refer to consumers, e.g., quality has been defined as "product performance that results in consumer satisfaction and freedom from deficiencies, and which avoids consumer dissatisfaction". Other definitions state that quality refers to product characteristics which "helps somebody and enjoys a good and sustainable market" or that "quality refers to the degree of standard of excellence, and/or fitness for purpose, and/or the consistency of attainment" of food properties. Quality has also been defined as characteristics of products "that bears on themselves ability to satisfy given needs" (Luning *et al.*, 2002). From these definitions, safety is indeed included in the consumers' needs and therefore in the quality concept. Furthermore, one major difficulty is that the political and/or the commercial organisations have made their own interpretation of what quality means from these different but complementary definitions. An added difficulty is that consumers' needs and wishes differ between countries (including within Europe) as discussed above.

Whatever the definition, most of the experts have also made a distinction between intrinsic and extrinsic quality attributes. The first refers to the product itself and includes for instance, (i) safety and health aspects, (ii) sensory properties (e.g. texture and flavour) and shelf life, (iii) chemical and nutritional attributes and (iv) reliability and convenience. The latter refers to traits more or less associated with the product, namely (i) production system characteristics (from the animals to processing stages including animal welfare, environmental aspects, and social considerations for instance), and (ii) marketing variables (including price, brand name, distribution, origin, packaging, labelling, and traceability) (Luning *et al.*, 2002; Grunert *et al.*, 2004). We will mainly consider intrinsic quality attributes in this paper, despite obvious strong interactions between both.

Some experts have also made a distinction between quality expectation and quality experience. Consumers form a quality expectation when they buy a product, but they form a quality experience upon consumption. Quality expectation determines, at least in part, consumer choice behaviour, while quality experience is of vital importance for repetitive purchase. Quality expec-

tation is assessed by quality cues which are related to intrinsic or extrinsic product characteristics. Quality experience depends on experience and credence quality attributes. The first are those that can be checked upon consumption (e.g. taste and texture), while credence attributes cannot be verified by the consumers (e.g. healthiness, animal welfare and environment concerns) (Luning *et al.*, 2002). Unfortunately, the number of quality cues and attributes included in this concept is increasing gradually, as well as the interactions between them, complicating research in this area. However, one clear goal of approaching the issue from a scientific point of view is to develop objective measurements of the different quality cues ranging from the impact of animal genetics, husbandry and physiology to the final product characteristics (Figure 1), and especially to convert credence attributes (which cannot be measured) into quality attributes, which can be objectively assessed.

Due to this complexity, a Total Food Quality Model was proposed in an attempt to integrate a number of approaches related to quality at the consumer end (consumer perception, intention of purchase and decision-making and consumer satisfaction) including the intention to purchase a product again, which in turn depends mainly on the relationships between quality expectation and quality experience before and after purchase (Grunert *et al.*, 2004). Because it is impossible to address all the parameters included in this model, only the most important ones among the intrinsic (colour, texture, flavour and juiciness and healthiness) and extrinsic (traceability) quality cues will be addressed in this paper. Attempts will be made in each section to explain why these cues are likely to be the most important ones. As a general example, focus group studies have shown that the strongest quality attibutes for beef are taste (flavour), tenderness, juiciness, freshness, leanness, healthiness and nutrition. Whereas before purchase, eating quality and healthiness have the same weight in quality expectation, eating quality has a stronger weight in quality experience during consumption (Grunert *et al.*, 2004). This emphasises the contradictory nature of consumers' wishes.

2. Sensorial quality

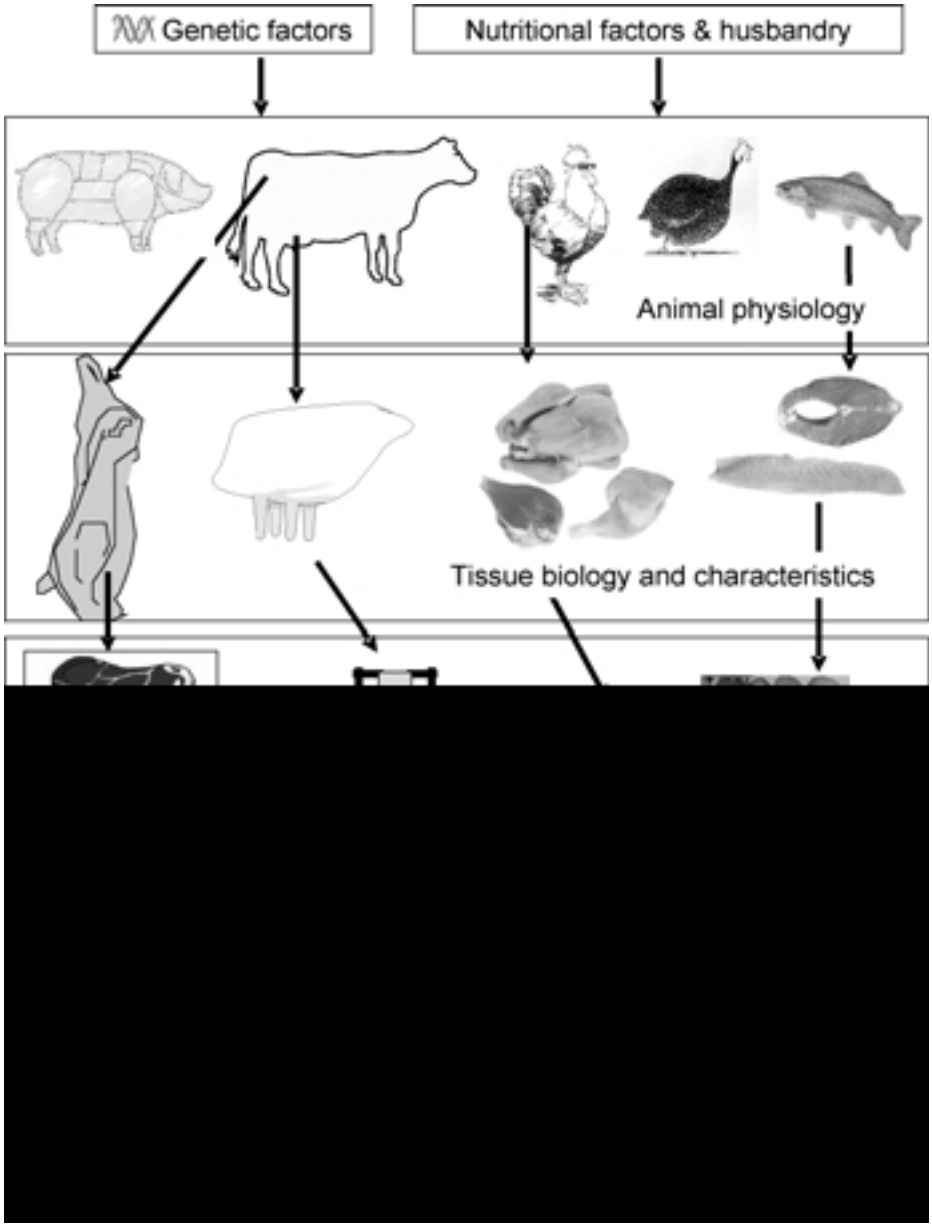
Colour

In meat and fish, colour is among the first quality attributes taken into consideration by the consumer to assess quality at the time of purchase. It is thus of utmost importance, especially in the case of salmonid flesh for which the orange-red colour is highly expected by the consumer.

Hence, there are different situations when we compare for instance meat and fish.

In meat, colour depends on the content and chemical state of myoglobin, the principal pigment naturally present in meat. Depending on the oxidation state of the haem iron, different myoglobin forms can be found. In the muscle, myoglobin is reduced and thus exhibits a purple colour due to the absence of oxygen. In contrast, at the surface of the meat, myoglobin is in contact with the air and therefore oxygenated (MbO) and it exhibits a bright red colour. However, prolonged storage with air induces oxidation and formation of metmyoglobin resulting in a brown colour unacceptable to the consumer. The pH of meat affects beef colour. A high pH indeed leads to a dark colour due to first a low scatter of light and second a very high rate of oxygen consumption combined with a slow rate of inward diffusion of oxygen into the muscle. This allows for a very narrow band of MbO at the surface and with the greater translucence, light penetrates further into the meat and more is absorbed, making it appear darker. More of that reflected has the appearance of Mb than MbO. This is a serious problem for young bulls which can be stressed easily, meat pH being higher in stressed animals at slaughter due to low muscle glycogen levels. Furthermore, the supplementation of ruminants with selenium and more particularly vitamin E can strongly reduce the rate of myoglobin oxidation (Geay *et al.*, 2001). Modified atmosphere packaging, containing high levels of oxygen to promote colour and carbon dioxide to inhibit bacterial growth, has extended the shelf life of retail meat cuts and allowed producers in many countries to distribute retail-ready packs over greater distances (Tewari *et al.*, 1999). Whilst the high oxygen can eventually impair quality and safety (e.g. rancidity and discolouration of meat)

Figure 1. Quality must now be considered as a convergence of consumers' expectations and needs with intrinsic and extrinsic quality attributes of food products depending on cultural habits. Today, consumers' choice and purchase depend on the interaction between economic and social contexts, their perception of quality traits (through quality labels) and the true quality traits of animal products. The latter depends on animal genetics, husbandry and physiology, tissue characteristics and the process of muscle and milk transformation into meat and dairy products. Safety and traceability must be ensured along the whole food chain. Quality and safety are now becoming interdisciplinary approaches with the aim to satisfy consumers.



this is usually not until well beyond the use-by date of the pack. The use of natural antioxidants can overcome some of these problems.

In salmonid flesh, the orange-red colour comes from carotenoid pigments, which must be provided in feed, since fish are not capable of synthesizing these pigments. There are many potential sources of carotenoid pigments: yeast, algae, krill or industrial derivatives such as crab and shrimp offal but these sources result in highly variable pigmentation due to the strong variability in pigment content and bioavailability. The main carotenoid used in aquaculture is astaxanthin sometimes in association with canthaxanthin (depending on EC and local regulations). However supplementation with these synthetic molecules increases the cost of feed by 15 to 30 %. Pigment fixation in the muscle depends on factors such as concentration of the pigment in the diet, level of dietary lipid, digestibility of pigment sources, duration of the supply and the aptitude of the fish to deposit pigments (Choubert, 2001). Deposition of astaxanthin in the muscle increases with the amount of ingested astaxanthin up to a plateau. Consequently, the colour of trout and salmon muscle then tends towards a maximum that cannot be exceeded. Choubert and Storebakken (1989) found that the maximal retention of astaxanthin was 12.5 % of the ingested dose in rainbow trout fed a dietary level of 25 mg per kg food. In the EU, the maximum recommended level is 100 mg of astaxanthin per kg of feed. In addition, since carotenoids are lipid-soluble compounds, their absorption is linked to that of lipids. Adding fat to the food favours pigmentation through an improvement of pigment digestibility. The effect of lipid sources on muscle pigmentation is still not clear. According to Choubert (2001), the concentration of astaxanthin in trout muscle would not be affected by the origin of the dietary fat (corn oil vs fish oil). As for meat, diet supplementation with antioxidants such as vitamin E and modified atmosphere packaging are tools to prevent pigment oxidation and changes in colour.

In conclusion, based on these two examples, depending on the animal physiology, the product characteristics and the consumer expectations, colour has to be controlled by different approaches including animal nutrition and management, processing and product conditioning. It is likely that the development of novel animal products due to innovative processing systems will induce new research in this area.

Texture and tenderness

Consumers ranked palatability and tenderness as the most important attributes of meat eating quality, and they can easily distinguish between tough and tender meat. The main source of consumer complaint is low and/or variable tenderness, and this is also the primary cause of failure to repurchase meat. This is particularly true for beef. Unfortunately, despite a great deal of research on the determinants of tenderness, little progress has been made, simply because the factors which affect tenderness are numerous and interact (Maltin *et al.*, 2003). Therefore, present research on tenderness aims to (i) clearly identify the factors and the causes of variation in tenderness, and (ii) identify indicators of tenderness to be able to predict tenderness and to develop methods to improve tenderness.

Factors and causes of variation in tenderness

Large differences in tenderness exist when comparing different muscles from the same bovine animals (Veiseth and Koohmaraie, 2005; Mullen and Troy, 2005). However, less variation, although significant, is observed when comparing the same muscle from different cattle breeds (Hocquette *et al.*, 2005a).

From a biochemical point of view, the main factors which determine ultimate tenderness are the background toughness and the tenderization phase.

From existing knowledge, it is clear that variation in the background toughness results mainly from the connective tissue characteristics which exist at the time of slaughter and do not change during the storage period. The indicators which are most often studied are collagen content and solubility. The high variability in collagen content across bovine muscles (from 1% to 15% of dry weight), and especially at the perimysial level, is likely to explain the major differences in toughness between beef cuts. Thus, connective tissue is considered to play a dominant role in toughness where its content is high. Therefore, any factors

related to the animal itself (e.g. genetic background, production system, physical activity or feeding) which has an impact on the collagen characteristics are widely studied. However, other factors (which remain to be extensively researched) are also important: e.g. thickness of perimysium, spatial distribution and composition of collagen, role of the different collagen isoforms, nature and role of the molecules which determine connective tissue organisation and/or which propagate tensional forces from muscle fibre sarcomeres to the connective tissue (McCormick, 1999; Purslow, 2005).

The tenderisation phase is characterised by a *post-mortem* proteolysis of muscle proteins. It occurs after the toughening phase caused by sarcomere shortening during rigor development. Different proteolytic systems have been shown to be responsible for the tenderisation process (Sentandreu *et al.*, 2002), but the calpain proteolytic system (with its inhibitor calpastatin) is thought to be mainly responsible for this process (Veiseth and Koohmaraie, 2005). During the early *post-mortem* phase, glycolysis also occurs bringing about the decline in pH of the muscle, and the rate of this decline is known to regulate proteolysis. Optimum tenderness can be reached within a few hours in chicken, 4 to 6 days in pork and lamb and 10-15 days in bovine muscles. This is associated with the muscle type which is fast-glycolytic in poultry at one extreme and slow-oxidative in cattle at the other. However, within the same species, the relationship between muscle tenderness and muscle type is highly complex and therefore controversial (Maltin *et al.*, 2003). But it was recently shown that slow-oxidative muscles of cattle tenderise as much as fast-glycolytic muscles whilst intermediate (fast-oxido-glycolytic) muscles are significantly tougher (Ouali *et al.*, 2005). This is the reason why a negative correlation was found between tenderness and the proportion of fast-oxido-glycolytic fibers in muscles, although tenderness was also found to be negatively correlated with fibre diameter (Dransfield *et al.*, 2003).

After slaughter, the extent of muscle contraction depends on the rate of the pH decline, residual ATP concentration and temperature of the carcass. Depending on the pH, muscle contraction may be high, when temperatures are low (<10ºC) or high (>35ºC) causing cold shortening or heat contraction respectively (Thompson, 2002; Savell *et al.*, 2005).

During cooking, a sharp increase in toughness of meat occurs between 40 and 50°C, and then above 65°C. Intramuscular connective tissue contributes to the tension development below 50°C. But its contribution is thought to decrease at higher temperatures and longer cooking times (Purslow, 2005). Others have suggested that the elasticity of whole meat varies with temperature thereby affecting the stress which has to be applied during mastication (Tornberg, 2005). Therefore, the different biological factors described above will act differently depending on the cooking method, and hence cultural habits of consumers.

Indicators of tenderness and methods to predict or improve tenderness

It is important for the beef industry to predict beef tenderness in the early *post-mortem* period as soon as the carcass becomes available in the meat factory. The indicators or the technologies employed so far include biological indicators (pH, colour) or methods to assess the ageing process, such as electrical impedance and conductivity. Some other methods are emerging such as ultrasonics, image analysis, fluorescence and near infrared spectroscopy, or immunoassays to assess proteolysis. Physical methods are widely used, but the correlation between shear force and tenderness depends largely on the breed and the production system (Mullen and Troy, 2005; Dufour, 2005).

For meat producers, it is also of interest for rearing or breeding purposes to predict the ability of live animals to produce tender beef. From a genetic point of view, a positive genetic correlation was found between marbling score and tenderness in the USA and Australia where beef toughness is assessed when cooked at high temperature (70°C). This relationship has driven most of the efforts dedicated to improving meat quality in these countries (Hocquette *et al.*, 2005b). At the molecular level, variants of several genes associated with the proteolytic process (the calpain 1 gene and the calpastatin gene) or collagen synthesis (the lysyl oxidase gene) have been shown to be associated with variations in tenderness. However, thanks to the

genome sequencing project and the development of high-throughput genotyping methods, it is likely that more genetic markers will be discovered in the near future (Kühn *et al.*, 2005). From a physiological point of view, it has been shown that tender beef has a lower proportion of fast-oxido-glycolytic fibres, slower *post-mortem* glycolyis, lower connective tissue and higher fat contents, which is to be expected based on the existing knowledge described above (Dransfield *et al.*, 2003). But only about one third maximum of the total variability in the final tenderness of beef could be explained by muscle characteristics (Renand *et al.*, 2001). It is commonly agreed that functional genomics will help to identify new genes/proteins and hence muscle molecules which have a significant impact on tenderness (Eggen and Hocquette, 2004; Hocquette *et al.*, 2005a). Some novel genes controlling muscle development (Sudre *et al.*, 2003) or associated with muscle growth potential (Sudre *et al.*, 2005) as well as proteins associated with muscle hypertrophy (Bouley *et al.*, 2005) or tenderness (Bouley *et al.*, 2004) have already been identified. A profile pattern of truly degraded protein would very likely be a more accurate tool to predict the final tenderness

We have seen that the tenderization rate of beef is particularly slow. This is the reason why it is important to mature beef for a sufficient period (about 10-14 days) to ensure sufficient tenderization and hence a high tenderness. This is highly recommended in France for beef sold with a quality guarantee. Similarly, products within the Meat Standards Australia (MSA) grading scheme cannot be sold to consumers before 5 days post-slaughter, and aging to 21 days increases the consumer score (Pethick *et al.*, 2005). Many leading retailers in the UK also specify a minimum ageing period for quality beef which must also have been electrically stimulated or hip suspended. This induces storage costs and related problems and this has therefore economic consequences for the beef industry. Another difficulty is that tenderization varies considerably between animals. As a result, it is difficult to ensure a consistent tenderness for consumers. Thus, predicting the final tenderness from information relative to the tenderization process is an active subject of research with the ultimate objective of marketing beef of a more constant tenderness. Some methods, such as electrical anisotropy (Lepetit *et al.*, 2002), have the potential to predict meat ageing.

Several tenderness enhancing technologies have been described (Tarrant, 1998; Culioli, 1999). For instance, some research is focused on muscle stretching for improving tenderness (Sorheim *et al.*, 2002). Now, the Tenderstretch method, or pelvic hanging, is widely used in some countries. Electrical stimulation is another method, which involves passing an electric current through the carcass of freshly slaughtered animals. This causes the muscles to contract increasing the rate of glycolysis resulting in an immediate fall in pH, an acceleration in the rate of proteolysis and an alteration in myofibrillar structure. It also prevents muscle from shortening excessively. But the challenge nowadays is to develop more effective electrical stimulation practices (Hwang *et al.*, 2003). On a whole, interventions at the early *post-mortem* stage remains a key issue.

Cooking method also affects the main factors which have an impact on the eating quality of meat, especially beef tenderness. Thus, cooking method was included in the input factors which drive the palatability prediction model of the MSA grading scheme, which has identified the major Critical Control Points (CCPs) from the production, pre-slaughter, processing and transformation sectors of the beef supply chain. Grilling (25 mm thickness) low connective tissue cuts results in high palatability scores. Roasting low connective cuts is equivalent to grilling. On the contrary, roasting gave a higher palatability scores than grilling for the high connective tissue cuts. Stir frying (10 mm) and thin slicing (4 mm) give similar results to grilling for low connective tissue cuts, but higher scores for the high connective tissue cuts (Pethick *et al.*, 2005). However, we must keep in mind that cooking methods depend largely on cultural habits and ways of life which differ between countries as discussed above.

In conclusion, meat tenderness is a complex function of production, processing and meat preparation. A guarantee for tenderness can only be given if all the factors affecting tenderness are controlled along the meat production chain.

Matching beef genotype, rearing system and processing system is a key challenge to ensure high and consistent tenderness at the consumer level. Modelling muscle characteristics for a beef product quality assurance scheme remains an important objective. The development of new further-processed more convenient-type beef products may change the priority of research in this area.

Flavour and juiciness

Tenderness is the most important eating quality attribute of meat in determining its acceptability, but when tenderness is increased, and variability decreased, then flavour and juiciness increase in relative importance (Love, 1994; Warkup *et al.*, 1995). Meat is aged (conditioned) to improve tenderness and flavour but longer conditioning under some packaging conditions produces 'bland' flavours (Spanier *et al.*, 1997). Ageing on the bone is thought to produce different flavours from ageing in vacuum pack, but bone-in meat aged in vacuum had higher beef flavour intensity than that aged on the carcass or boned out and aged in vacuum (Jeremiah and Gibson, 2003). However, in other studies, vacuum storage produced few significant differences (Campbell *et al.*, 2001) and dry ageing produced consistent positive effects on flavour (Warren and Kastner, 1992). Juiciness is more difficult to quantify and is sometimes combined with tenderness to produce the term 'succulence'. Speculation continues as to the role of fat, particularly marbling fat, in juiciness and flavour. It is the key criterion of the USDA Quality Grade for beef, but Dikeman (1987) concluded that there is only a small positive correlation with tenderness, juiciness and flavour scores and the range of fat levels required to produce significant sensory differences in juiciness or flavour are large compared to modern production practices. Recently, positive genetic correlations were found for juiciness and flavour. Fat and lean lines of lamb, scanned by X-ray computer tomography, differed significantly in muscle density, intramuscular fat and juiciness, as assessed by a trained sensory panel (Karamichou *et al.*, 2005).

It is generally held that the flavour of meat resides in the water-soluble fraction, whereas species-specific flavours are located in the lipid soluble fraction (Mottram, 1998). However, diets such as forages or cereal, which can dramatically change the fatty acid composition of the meat (see following section), can also change the flavour (Elmore *et al.*, 1999a; Elmore *et al.*, 2004). Feeding linseed oil and/or fish oils to beef and lamb produced a higher muscle content of n-3 polyunsaturated fatty acids (PUFA) (18:3*n*-3; 20:5*n*-3; 22:6*n*-3) (Scollan *et al.*, 2001; Cooper *et al.*, 2004). With fish oil in particular, the meat produced was oxidatively more unstable with more abnormal, fishy and rancid flavour notes (Vatansever *et al.*, 2000) and produced many more lipid degradation products upon cooking. Compounds such as n-alkanals, 2-alkenals, 1-alkenols and alkyfurans increased up to 4-fold and most of these compounds were derived from autoxidation of mono- and di-unsaturated fatty acids and were promoted by increased levels of PUFAs (Elmore *et al.*, 1999b). In a similar study comparing grass- and concentrate-finished animals, the biggest difference was that the concentrate-fed animals had higher concentrations of linoleic acid in their meat and on cooking produced seven compounds at over three times the level found in meat from grass-fed animals, which had much higher concentrations of α-linolenic acid and produced a higher amount of only one compound, 1-phytene (Elmore *et al.*, 2004). Lorenz *et al.* (2002) quantified meat odour volatiles formed after pressure-cooking meat produced by feeding beef animals forage or concentrate. 'Green' odour from meat of grass-fed animals was connected with compounds (hexanals) derived from oleic (18:1*cis*-9) and a-linolenic acid (18:3*n*-3), and "soapy" (octanals) from linoleic acid (concentrate fed). Campo *et al.* (2003) used a trained sensory panel to study the flavour of the individual fatty acids 18:1, 18:2 and 18:3, alone or in combination with cysteine and/or ribose. Meaty aromas were much more pronounced when cysteine and ribose were present, i.e. interactions between Maillard reaction products and fatty acids. The three fatty acids produced different odour profiles and 18:3 in particular produced high scores for fishy and linseed/putty. When cysteine and ribose were present 'grassy' was more prevalent especially in the presence of $FeSO_4$ as pro-oxidant.

These studies could explain the differences

seen by sensory panels for forage- and feedlot-(cereal) fed beef and lamb (Wood *et al.*, 2003), but they do not explain the differences for flavour preference seen in different countries. Whilst this may be due to familiarity with a certain flavour (vide infra) there are other explanations. In a US study, Larick *et al.* (1987) observed that steers finished on white clover (*Trifolum pratense*) had a higher "grassy" flavour than those fed grass (*Festuca arundinacea*) and range-fed animals produced meat which was not only higher in PUFA but was also oxidatively more unstable (Larick and Turner, 1990). The authors attributed these flavour differences to both the increased content of PUFA, particularly α-linolenic acid in the phospholipid fraction, and its lower oxidative stability. Oxidation products of fatty acids probably accounted for the abnormal, unacceptable cooked flavours. Other studies have found it more difficult to distinguish flavour differences in grain - vs. concentrate-produced beef meat. Moloney *et al.* (2001) suggest that this may be due to other factors than the fatty acid composition, such as the antioxidant content. In European studies it has been confirmed that that feeding fresh, green forages not only promotes the content of PUFA in meat, but also contributes antioxidants such as α-tocopherol and β-carotene to the meat, which stabilise the fatty acids and make the meat more desirable (Richardson *et al.*, 2004; Gattellier *et al.*, 2005).

Within more extensive ruminant production systems, type of forage has been associated with pastoral flavour in ruminant lean and fat (Young *et al.*, 2003). The effect of type of forage on flavour is better documented for sheep. Lambs grazed on white clover or alfalfa had more intense flavour than those grazed grass pastures whilst lambs finished on grass pasture at a slow rate of growth had more intense flavour than those finished on pasture at a high rate of growth or on concentrates (Duckett and Kuber, 2001; Roussett-Akrim *et al.*, 1997). Whilst the odour/flavour of lamb is due to a wide range of compounds the characteristic species flavour is caused by branched-chain fatty acids (BCFA), resulting from propionate produced in the rumen, and odour from skatole (3-methylindole), produced by degradation of tryptophan in the rumen, particularly the faecal and barnyard notes associated with those raised indoors (Young *et al.*, 2003). Culture and meat consumption habits determine its acceptance with BCFAs being more responsible for Japanese consumers disliking of sheepmeat than skatole (Prescott *et al.*, 2001). Skatole is also found in beef, particularly animals finished on grass or conserved forages, though its role seems to be less important in beef than sheep. Recent data suggests that skatole is not a negative contributor to flavour as it is for example in pork meat (Lane and Fraser, 1999; Whittington *et al.*, 2004). Skatole has been identified as the faecal taint in milk from cows fed *Lepidium* spp. (Park, 1969).

It is of note that studies on milk and cheeses (e.g. Saint-Nectaire-type in France) have shown that changes from pasture to concentrate feeding will produce paler, whiter, less well appreciated butters and cheese. Differences in cheese flavour were noted between winter and summer production, between high mountain and lowland pastures and even from north and south facing slopes (Coulon *et al.*, 2004).

The role of breed in flavour development is contentious. Breed effects on eating quality, particularly flavour, are usually small and where they exist are related to differences in fatness, with fatter animals generally tending to have higher flavour intensity scores (Laborde *et al.*, 2001; Chambaz *et al.*, 2003). It is often assumed that the traditional beef breeds, such as Aberdeen Angus, have the best flavour and dairy breeds the worst although there is little scientific evidence for this. In one study, no difference was found between Holstein-Fresian steers and Welsh Blacks (Vatansever *et al.*, 2000), whilst in another, Koch *et al.* (1976) found that Jersey and South Devon crosses produced more tender and juicy meat than that from traditional beef breeds. This is possibly because in dairy breeds taken to the same level of finish, intramuscular fat is higher. Martinez-Cerezo *et al.* (2005) compared three Spanish breeds (Rasa Aragonesa, a local meat breed, Churra, a dairy breed and Spanish Merino, a specialised meat breed) at different slaughter weights and after different periods of aging of the meat in vacuum pack. Lamb odour and intensity increased with slaughter weight, with the highest score for Rasa Aragonesa. Off-flavour was highest

for Rasa Aragonesa and increased with ageing times. Juiciness was influenced by breed, age and conditioning, with the lightest Churra and heaviest Spanish Merino producing the juiciest meat.

In lambs, diet appears to have a greater effect than breed and differences in flavour, explained by higher concentration of 18:3 in animals fed grass and 18:2 in animals fed concentrates, appear to be more intensive in lamb. Cross-bred Suffolk's finished on lowland grass or concentrates were compared with Soay, finished on lowland grass, and Welsh Mountain finished on upland flora (Fisher *et al.*, 2000). The grass-fed animals showed typical fatty acid compositions with elevated 18:3n-3 and long chain polyunsaturated fatty acids (PUFA), but Soay had more than double the amount of 18:2n-6 than other grass-fed breeds and the same as the Suffolks fed concentrates, despite being less fat overall. Flavour characteristics were similar for lamb chops from Welsh Mountain and Suffolks fed grass, which differed from Soay and Suffolks fed concentrates. The latter had low scores for flavour and high scores for abnormal flavour, metallic, bitter, stale and rancid. Soay also had a high score for livery, sometimes scored highly for beef, and may be related to its leanness, darker colour and higher myoglobin content.

Flavour is not only a desirable attribute of meat, but its particular nuances must be familiar to the consumer. The UK exports carcasses from small breeds of lamb to Spain where carcasses are often obtained from early-weaned milking breeds, but these UK carcasses will be older, grass-finished and of stronger flavour. Whilst British and Spanish panels described Welsh and Spanish lamb meat to the same order of magnitude for texture and strength of flavour, the panels showed preference for familiar flavours. The Spanish panel preferred Spanish lamb and the English panel, English lamb (Sanudo *et al.*, 1998). A similar conclusion was drawn when Japanese and New Zealand consumers tested New Zealand lamb (Prescott *et al.*, 2001). Results are much clearer than those found for beef. If members of the EU are to trade in meats on the world market; then not only must the quality of such products in terms of texture, juiciness and flavour, be of a high standard, but flavour, in particular, from their production systems must be familiar or acceptable to the flavour preferences of consumers in the target market.

3. Nutritional value

Healthiness of human beings depends on the balance between dietary intake and quantitative and qualitative needs of individuals. Obviously, healthiness is linked to the quantitative amount of ingested food products and their individual nutritional value. This is the reason why some food products are suggested to be used in moderation only despite their high sensory value or low price for instance. This is also the reason why many countries (such as the USA, Italy, Scandinavian countries, Germany in association with Austria and Switzerland, the United Kingdom, France, the European Union, etc) have produced nutritional references to assess the nutritional quality of current dietary intakes of populations, to determine their adequacy for physiological requirements, depending on age, physiological status, physical activity, etc, or to modify existing diets and food products and even to conceive novel diets of food products to satisfy these requirements.

Nutritional quality of food per se is related to its nutrient content, their bioavailability and their ability to meet the human requirements. For instance, fish flesh has a high nutritional value because it contains highly digestible protein (17 – 22 g/100 g wet weight) with a well balanced amino acid profile, it is also rich in free amino acids (1.3 to 3.8 g/100 g) and low in collagen (3 to 10 g/100 g compared to 16 to 28 g/100 g in beef meat) and supplies a variety of minerals, vitamins and essential fatty acids (Médale *et al.*, 2003). Traditionally, meat and milk have also been considered to be highly nutritious and valued foods. The importance of meat as a source of high-value protein is well accepted. For instance, all muscles have a similar amino acid profile (except those rich in connective tissue) which is not far from the human's requirement (Culioli *et al.*, 2003). Similarly, the milk proteins are of high nutritional quality due to their favourable composition in essential amino acids (Lucas *et al.*, 2005). This is of utmost importance because the major portion of dietary proteins comes from animal products (65%

in France in 1995, half by meat and meat products, 35% by dairy products and 8% by fish and sea products). Furthermore, the digestibility of these proteins is high compared to plant proteins. Additionally meat, fish and milk are recognised as important sources of micronutrients (including for example calcium, immunoglobulins, bioactive peptides, sphingolipids, vitamins A, B_6, B_{12}, C, D, E, iron, zinc and selenium).

However, a number of negative factors (such as high fat and saturated fat content, associations between red meat and cancer and non-nutritional issues such as animal health scares, BSE, foot and mouth disease) have played down the many positive nutritional attributes of meat and milk. In particular, health concerns have been targeted at the fat content and fatty acid composition of meat and milk. Fat in these raw food materials provides essential fatty acids and vitamins to the consumer and for example, in meat, fat plays an important role in the sensory perception of juiciness, flavour and texture (Wood *et al.*, 2003). However, there is a perception among consumers that meat (in particular red meat) and milk are foods with a high fat content rich in saturated fatty acids (SFA) which is considered to contribute towards certain human diseases. Finally, while the protein level and amino acid profile of fish and meat are nearly constant, the micronutrients content, lipid level and fatty acid composition are greatly affected by feed composition and rearing factors of the animals. This emphasises the responsibility of agricultural practice in providing animal products of high or low nutritional value.

In addition, consideration must be given to the target consumer base when considering routes for exploiting research about nutritional value of food. The concept of "functional" foods has increased in recent years. The term functional foods is a generic term used to describe foods or food components that have beneficial effects on human health above that expected on the basis of nutritive value (Milner 1999). Such products are targeted at disease prevention and are aimed at healthy people. Omega-3 and/or CLA enriched products would make a valuable consideration in this respect and indeed in some countries the dairy industry has been progressive in developing such products. The problem with this approach is that it will only target a limited consumer base and largely those who can afford to pay for such products. However, in terms of health maintenance and disease prevention in the wider consumer base, it is more appropriate to consider application of this research to improving the healthiness of ruminant products in general.

Recent guidelines from the World Health Organisation and the Food and Agriculture Organisation emphasise the importance of maintaining a balanced diet in helping to reduce the incidence of non-communicable diseases such as obesity, type-2 diabetes, cancer and cardiovascular disease (WHO, 2003). For fat it is recommended that total fat, SFA, n-6 polyunsaturated fatty acids (PUFA), n-3 PUFA and trans fatty acids should contribute < 15-30, < 10, < 5-8, < 1-2 and < 1 % of total energy intake, respectively. Within these targets reductions in the intake of SFA (which are known to raise total and low-density lipoprotein cholesterol) and increases in the intake of unsaturated fats (in particular the omega-3 PUFA which are known to be beneficial to in human health and disease prevention; Simopoulos, 2001; Leaf *et al.*, 2003) are important issues.

The total fat content of raw meat and milk typically ranges between 1-8 g/100 g food and is influenced by a variety of factors including for example animal breed, diet, age. However, the fat content of meat and dairy products ranges considerably from typically 1-5% in raw muscles depending on the species (chicken, pigs, cattle) (Culioli *et al.*, 2003), 10% in low fat sausages, 20-35% in most cheese varieties (Lucas *et al.*, 2005) to 40-50% in salami. Ruminant fat typically contains a high proportion of SFA (40-60 %; largely as a consequence of microbial biohydrogenation within the rumen) and monounsaturated fatty acids (MUFA; 30-50%) and small amounts of PUFA (5%). Oleic acid (18:1n-9) is the most prominent MUFA, with the remainder of the MUFA occurring mainly as *cis* and *trans* isomers of 18:1. Linoleic (18:2n-6) and α-linolenic acids (18:3n-3) are the main PUFA. Generally, the PUFA and MUFA are regarded as beneficial for human health and there is even recent evidence of positive effects of 18:1 *trans*-11 (Corl *et al.*, 2003), though other work suggests negative effects (Clifton *et al.*, 2004). The predominant SFA are

14:0, 16:0 and 18:0. As mentioned above, there are concerns about the effects of SFA on plasma cholesterol, though 18:0 is regarded as neutral in this regard. Myristic acid (14:0) is regarded as more potent than palmitic acid (16:0) in raising plasma lipids. Meat and milk products from ruminants are also the main dietary sources of conjugated linoleic acid (Ritzenthaler *et al.*, 2001), which have being identified as processing a range of health promoting biological properties including anticarcinogenic activity of the dominant CLA in milk and meat, the *cis*-9, *trans*-11 isomer. Poultry and pork contain higher levels of unsaturated fatty acids and PUFA than ruminant products (Wood *et al.*, 2003).

In most European countries, the consumption of higher levels of total SFA and fat intake is associated with meat and milk products (Valsta *et al.*, 2005). This has contributed towards a more negative image for these products. However, meat contains significant amounts of beneficial *n*-3 PUFA, particularly α-linolenic acid (18:3*n*-3) but also the long chain PUFA, eicosapentaenoic acid (EPA; 20:5*n*-3) and, docosahexaenoic acid (DHA; 22:6*n*-3). This is the reason why a great deal of research has been conducted to manipulate the fatty acid composition of meat. Higher amounts of EPA and DHA are commonly found in fish and sea products.

In fish, numerous studies have demonstrated that the fatty acid profile of the diet has a strong impact on the fatty acid profile of the lipid deposited in muscle. However the variation in flesh fatty acid content is typically less pronounced than that of diet (Bell *et al.*, 2003; Régost *et al.*, 2003a). As aquaculture consumes a large proportion of the fish oil available on the market, alternatives to fish oils in fish diets have received considerable attention in recent years, but the fatty acid composition of vegetable oils differs considerably from that of marine fish oils. Substitution of fish oil by vegetable oils in the feed dilutes EPA and DHA levels in fish flesh and increases the content in 18:2*n*-6 with consequences on taste, healthy values and consumer acceptance. A promising feeding strategy to reduce the use of fish oil for aquaculture without compromising the fatty acid profile of fish flesh is the return to a fish oil diet some weeks prior to slaughter (Bell *et al.*, 2003; Régost *et al.*, 2003a; Régost *et al.*, 2003b). According to an ongoing study performed in the scope of the European research project RAFOA (Corraze *et al.*, personal communication), feeding rainbow trout, previously fed for 9 months on a 100% vegetable oil (rapeseed or linseed oil), on a fish oil diet for 3 months restored 80 % of DHA and EPA but wash out of 18:2*n*-6 was less efficient. Further research is needed to find the right balance in order to produce fish with the desired fatty acids profile.

Despite the great interest of fish products, meat and milk are, however, a significant source of *n*-3 PUFA for many people since consumption of oily fish is low (British Nutrition Foundation, 1999). Research has focused much attention on methods of enhancing the nutritional value of milk and meat by further increasing *n*-3 PUFA and CLA (Lock and Bauman, 2004; Scollan *et al.*, 2005; Wood *et al.*, 2003). The main approach employed in these studies is based on modifying the animal diet by enhancing the intake of MUFA and/or PUFA provided as (1) fresh and ensiled forages (i.e. grass), (2) oils and oilseeds (i.e. sunflower, linseed), (3) fish oil and marine algae. In ruminants, because dietary lipids are extensively modified in the rumen, this research is contributing towards an increased understanding of the relationships between diet, rumen fermentation, lipid metabolism and incorporation of beneficial fats into lipid in muscle and milk. The fatty acid composition of meat and milk products have also been modified during processing by adding vegetable oils leading to improvement in nutritional value (Valsta *et al.*, 2005). Both these approaches help to improve the nutritional value of meat and milk and it is important that efforts continue to seek consumer acceptable approaches for further improvements. This work has contributed towards an increased awareness that these foods do contain micro-components which may confer beneficial effects on health maintenance and disease prevention.

Among the lipid soluble vitamins, tocopherols are of major interest because their main function is to protect tissues unsaturated fatty acids against oxidation by blocking the free radicals reaction chain. Oxidation of fish lipids is a major problem of flesh quality due to its high content of PUFA which are very sensitive to oxidation: it can

decrease the nutritional value of the product by lowering the PUFA content, producing off-flavour compounds and modifing texture and colour by oxidizing pigments. Several studies conducted in different fish species demonstrated that deposition of lipid-soluble vitamins such as vitamin E in the muscle was dependant on the dietary supply (Frigg *et al.*, 1990; Bai and Gatlin, 1993; Sigurgisladottir *et al.*, 1994). Fish muscle enriched in vitamin E by increasing dietary tocopherol level has an improved oxidative stability during storage (Frigg *et al.*, 1990; Bai and Gatlin, 1993; Baker, 2001). The main source of aroma in fish being the compounds formed by the oxidation of PUFAs, increasing the concentration in vitamin E in the feed leads to a reduction in the "fishy" aroma of fish fed high fat diets (Chaiyapechara *et al.*, 2003). Similarly, lipid oxidation is also a problem during storage of meat or milk fats. For meat, lipid oxidation induces unpleasant tastes and odours and is associated with changes in colour, and with the potential formation of toxic compounds. For milk, spontaneous oxidised flavours develop when fats are oxidised. Fat oxidation is reduced by the presence of endogenous antioxidative enzymes present in milk and meat, and also by antioxidants (such as lactoferrin, vitamins C and E and carotenoids in milk, or vitamnin E and histidine-containing dipeptides (i.e. carnosine and anserine) in meat). New strategies in terms of antioxidant supply should be developed especially when animal products are enriched in PUFA (Durand *et al.*, 2005).

4. Safety

The importance of safety as a food attribute has increased significantly in recent years and the integrity of the food chain is now a priority for the agri-food sector. Microbial infections related to foods of animal origin, alone impose a substantial public health and economic burden on society. In addition, there are concerns about the long term effects on public health from exposure to chemical residues though the food supply and despite current controls related to removal of spinal cord and associated tissue there remains fears about exposure to prion material. This section will focus on safety issues related to microbial contaminants only.

Primary production

It is well documented that many potentially harmful pathogens (*Salmonella*, Verocytotoxigenic *E.coli* in particular *E. coli* O157:H7, *Listeria monocytogenes, Campylobacter, Cryptosporidium parvum* and others) can be carried by animals and shed in their faeces. In the environment, not only in faecal material but also in the underlying soil and grass, many pathogens survive for extended periods ranging from several weeks to many months. This provides an important transmission route for pathogens within herds, farms, the fresh food chain, water courses and the wider environment. Some farm management practices have been shown to affect the general carriage and spread of pathogens. In particular, the provision of clean (non-contaminated) feed and water and reduction in stock, housing and grouping densities will reduce the prevalence of pathogens within herds. Manipulation of diet pre-slaughter can alter the gut flora and can affect rates of faecal shedding (Van Baale *et al.*, 2004). Alternative future strategies to reduce the prevalence of pathogens in food animals could involve immunisation with a view to preventing colonisation and carriage (Potter *et al.*, 2004) or the incorporation of probiotics into animal feed (Schamberger *et al.*, 2004). Undesirable faecal shedding of pathogens by all animals is increased by transport stress and is a particular problem if animals are transported long distances in confined spaces or held in lairage for prolonged periods.

Salmonella is the second most common cause of bacterial food borne illness and pigs are recognised as an important vector. Recognising this, many EU countries have national control programmes for *Salmonella* in pig herds based on establishing *Salmonella* status of herds. An ELISA method is used to tests for antibodies to *Salmonella* in the meat juice at slaughter and results in a herd categorisation status (1-3) based on the percentage of the herd which test positive. A positive test indicates that the pig has been exposed to *Salmonella* at some point within the last two months and provides the starting point for further investigations and either specific measures are taken at slaughter or specialist advice is given to farmers about measures to control *Salmonella* on-farm.

Slaughter

Pathogens maybe present in the gut and faeces of food animals presented for slaughter. During the slaughter evisceration process, these enteric organisms may be transferred to carcasses from the intestines, stomach contents, oral cavity or oesophagus. The risk of faecal contamination on the carcass at this stage of slaughter can be reduced by specific procedures including "rodding" (a technique used to separate the oesophagus from the trachea and diaphragm). Bagging and tying of the bung can also help prevent contamination of the carcass. In addition, gut micro-organisms in the faeces can contaminate the hide (cattle) or fleece (sheep) and it is generally accepted that the amount of faecal material adherent to animals delivered to abattoirs significantly influences the levels of microbial contamination on derived carcasses. Removal of hides should be carried out in a manner that avoids contact between the hide and the carcass. This can be achieved by a number of measures including the use of hide pulling equipment and using clean equipment (immersion of knives in water at 82°C) for the dehiding operation.

Because of the risk of transmission of *E. coli* O157:H7 from cattle faeces and hides, many EU countries have implemented "clean cattle policies", which aim to reduce the level of contamination on carcasses and derived raw meat products. The level of faecal material on the animal hide is judged by visual *ante-mortem* inspection. Subsequent strategies for the processing of dirty animals may include the rejection of animals with excessively dirty hides, washing of the animals, hide trimming or clipping, slaughter of dirty animals at the end of the kill period or reducing the speed of the slaughter line.

Intervention steps used to decontaminate carcasses include cold (10-15°C), warm (15-40°C) or hot (75-85°C) water washing, organic acid sprays (acetic, lactic), steam pasteurisation or combinations of these procedures. While organic acids are widely used in the USA, they are not permitted under EU regulations for beef carcass decontamination.

Meat products

The comminution of whole raw meats to produce burgers, steak tartar etc., distributes the initial surface contamination throughout the derived product. Commonly used additives for raw meat such as 3% sodium lactate, have no significant effect on the survival of *E. coli* O157:H7. Therefore the primary control measure remains adequate cooking of the product or stringent hygiene in the case of streak tartar. Fermented meat products are normally rendered microbiologically safe by a combination of factors including pH reduction, end products of fermentation and a range of other additives. *E. coli* O157:H7 have a greater ability than other enteric organisms to survive the low pH of such products and pose a particular risk. Concerns in relation to the survival of E. coli O157:H7 have led to the recommendation that the processes for such ready to eat meats should achieve a $\log_{10}5.0$ cfu/g decline in numbers of this pathogen. It is difficult to manipulate the intrinsic factors in the fermentation process to achieve this target and additional hurdles in the process such as the inclusion of a heat treatment step are most successful (Riordan *et al.*, 2000).

Dairy products

Many cases of microbial infections have been linked to the consumption of raw milk and derived products. Pasteurised milk and dairy products have been implicated in human infections but only where inadequate pasteurisation or post process contamination was reported. In hard cheeses, the additional process hurdles including the low water activity and pH as a result of the curing process, and the differences in the competing microflora reduces the potential for survival or growth of pathogens in this product compared to high moisture soft and semi-soft cheeses. *Listeria monocytogenes* and *E. coli* O157:H7 in particular pose a risk in soft unpasteurised cheeses (Sanaa *et al.*, 2004) and immumocompromised people are advice not to consume such products.

Regulatory issues

Following the publication of the White Paper on food safety in 2000 the EC carried out a major review of the hygiene directives. The numerous directives developed ad hoc since 1964 intermingled different disciplines, including hygiene, ani-

mal health and official controls. The existence of different hygiene regimes for products of animal origin and other food have led to a detailed and complex regulatory situation. Discussions and negotiations on new legislation, generally referred to as the Hygiene Package (H1 to H5) have been on-going for a number of years and are now being finalised. This new hygiene legislation, applicable as of January 1st 2006 will be more streamlined. Hygiene 1 sets out the rules applicable to all food businesses from farm to sale to the consumer and places primary responsibility for the safety of food on the food producer. Hygiene 2 details specific hygiene rules for food of animal origin. Hygiene 3 details the official controls for products of animal origin and H4 sets down animal health rules for food animals. Hygiene 5 repeals the 17 existing directives while leaving the implementing decisions in force. Issues within these new directives which will significantly impact on the food sector include HACCP being legally mandated for all food business and changes to microbiological criteria for fresh foods including a new requirement to test for *Salmonella* on fresh meat species.

It is and will remain for the forseeable future a challenge for global, EU and national policy makers, the regulatory authorities and the food industry to reduce the burden of microbial food borne illness from both know contaminants and those which are emergent or may emerge in the future. It is now well recognised that the best way to address this challenge is to design food safety management systems based on the principles of risk analysis linking an acceptable public health risk to food safety objectives and set microbial criteria for the food producer to achieve.

5. Traceability

Traceability is an important component of quality policy in agribusiness. It is defined by the international standard ISO 8402 as 'the ability to trace the history, application or location of an entity by means of recorded identifications'. This definition covers both the origin of the entity, i.e. traceability back to the origin, and the mode of production of this entity, i.e. traceability of the processes. Traceability back to the origin refers to an animal's identity, breed and geographical origin, which are often important considerations for quality labels. Traceability of process refers to production systems, including feeding diets, processing and conservation processes and any adulteration of products. Traceability was initially mandatory only within the general framework of the certification of protected denomination of origin (PDO) status, and was used to differentiate niche market products. However, since the BSE crisis, it has also been used to implement food safety. There is currently an increasing consumer demand for information and guarantees concerning the mode of production of animals, and particularly animal feeding diets. Bodies operating product certification systems also require control tools, to be able to guarantee objectively that specification commitments have been fully met. Being able to trace the feed given to animals is therefore a major challenge for scientists, monitoring and commercial entities and farmers. This section deals mainly with traceability of feeding diet, and also gives some insight into traceability of geographical origin, but see San Cristobal-Gaudy *et al.* (1999) for traceability of animal's identity and Rouzaud *et al.* (2000) for traceability of animal's breed.

Potential tracers and approaches

Efforts have recently been made to develop analytical tools to quantify specific compounds in the product, or the animal tissues and fluids that can act as tracers of the animal's feeding diet. Four approaches have been investigated to provide information on the feed given to herbivores: (i) plant biomarkers, coming directly from the diet, (ii) metabolic markers derived from animal metabolism, (iii) physical markers, and (iv) global approaches. Plant biomarkers such as carotenoids and terpenes, animal metabolites such as 2,3-octanedione, skatole, fatty acids and ratios of oxygen-, carbon- and nitrogen-stable isotope, are potential tracers in meat and milk or animal tissues, of animal feeding diets. Phenolic compounds also recently proved their usefulness in successfully discriminating milks obtained from cows fed different diets (Besle *et al.*, 2005) and an experimental evaluation of these compounds for meat is warranted. Terpenes, phenolic compounds and

ratios of stable isotope are also potential tracers of the geographical origin of milk and meat. Full details of the corresponding analytical methods can be found in Prache *et al.* (2003a; 2003b) for carotenoid pigments, in Besle *et al.* (2005) for phenolic compounds, in Priolo *et al.* (2004) and Cornu *et al.* (2005) for volatile compounds (terpenes, 2,3-octanedione and skatole), in Piasenter *et al.* (2003) and Renou *et al.* (2004a; 2004b) for the isotopic composition of the water and fat of tissues and products and finally in Loor *et al.* (2005) and Aurousseau *et al.* (2004) for the fatty acid composition of dairy products and of the meat.

Global approaches, especially near infra-red spectroscopy (Cozzolino *et al.*, 2002) and functional genomics (Cassar-Malek *et al.*, 2005) are just emerging and need further experimental evaluation.

Some leading examples and results in meat and dairy products

These techniques have already allowed the discrimination of products obtained in contrasting feeding conditions. In dairy cows, a research project of the INRA Research Centre of Clermont-Ferrand/Theix has been developing analytical techniques to distinguish animal feeding diets in milk and cheese (Martin *et al.*, 2005). Terpene profiles from dairy products (milk and cheese) proved effective in identifying the presence of diverse grassland types in the feed. Beta-carotene content or measurement of the reflectance spectrum of milk and cheese can distinguish 'maize silage' or 'hay and concentrate' products from 'grass silage' or 'grazed grass' products. Milk fatty acid composition gave valuable information to single out the concentrate-rich diets from the others. Nevertheless, those individual compounds offer only a partial solution in distinguishing one diet from another. In a trial where cows were fed with six different diets (rich in concentrates -65% of the diet-, based on maize silage, ryegrass silage, ryegrass hay, native mountain hay and native mountain grazed pasture), the best results were obtained by combining analyses of terpenes compounds and fatty acids, which allowed successful categorization of 100% of the milk.

In lamb meat, we have undertaken at the INRA Research Centre of Clermont-Ferrand/Theix a project to find tracers for grass-feeding in lamb (Prache *et al.*, 2005). We compared lambs fed either exclusively grass at pasture or a diet containing 85% concentrate and 15% hay indoors (besides maternal milk for both groups). The feeding level of stall-fed lambs was adjusted to achieve similar growth patterns in both treatments until slaughter. In these studies, carotenoids in the blood and in the perirenal fat, terpenes and 2,3-octanedione in the fat, and fatty acid composition of the meat all made it possible to distinguish between grass-fed and stall-fed animals. Grass-fed lambs had 5 to 6 times more carotenoids in their blood, 2.4 to 4.1 times more lutein in their perirenal fat (Prache and Theriez, 1999; Priolo *et al.*, 2002; Prache *et al.*, 2003a; Prache *et al.*, 2003b) and 25 times more 2,3-octanedione in their fat than stall-fed lambs (Priolo *et al.*, 2004). The (*n*-6)/(*n*-3) ratio in total lipids was tripled in concentrate-fed lambs compared with the grass-fed lambs (Aurousseau *et al.*, 2004).

In beef cattle, the French production system 'Bœuf Fin Gras Du Mezenc', which qualifies for PDO status, is committed to keeping the concentrate consumption below a set limit (700 g/100 kg liveweight per day). It has recently been demonstrated that the spectral characterization of the faeces using NIRS can be used successfully to control this specification commitment (Noziere *et al.*, 2005). Six heifers were fed 2, 4 or 6 kg concentrate/day (+ hay so that total feed per day was 9 kg) in a latin-square design. After a two-week adaptation period, samples of faeces were taken every 6 h over a 48 h period to account for maximal variability, and scanned in a NIRS instrument in a reflectance mode. Principal component analysis of optical information showed differences in faeces resulting from the different diets, that permitted satisfactorily authentication that the specification commitment has been fully met.

In conclusion, these examples indicate that it is possible to determine the diet which herbivores have received using analytical methods that quantify direct or indirect tracers in the product or the animal tissues. Results also show that the combined use of different tracers and tissues may be useful. These methods were however developed using a relatively small number of animals and

they are currently being validated with a much larger number of animals. A validation procedure is essential because between-animal variability can be high, and the ability to accumulate potential tracers may include a genetic component. Further research is also directed at elucidating the sources of variation of the animal's response and the quantification of their effects, studying the latency of appearance and persistence of tracers in the case of modification of animal diet (Prache *et al.*, 2003a; Prache *et al.*, 2003b; Noziere *et al.*, 2004), and addressing the potential sources of bias and fraud.

General conclusion

Since consumers' expectations are the basis of the quality definition, food quality is more and more a social concept, although biological sciences are essential to characterise animal products, and technical sciences to improve quality attributes (Figure 1). Fundamental research is also important to understand the underlying biological and physical mechanisms and to suggest new techniques with a quality improvement.

Eating quality is an important determinant of food choice. If the product does not look attractive, does not smell good or does not taste good, the consumer does not purchase it despite its nutritional and health value. Therefore, sensory quality remains a research priority. Generally, the question of sensory quality (for instance colour and tenderness for meat) must now be studied by systemic approaches due to the complexity of the problem. Research must continue to focus on methods of improving the nutritional value of fish, meat and milk and associated products. This work helps to provide novel approaches which may contribute to product differentiation in the market place, helping to add value across the food chain. Consumer communication is also essential to correct consumer and medical myths and to increase awareness of new information on the enhanced properties of a wholesome food.

The importance food safety has been considerably increased during the last decade due, at least in part, to recent crises such as BSE and foot and mouth disease. In reality, the incidence of these problems in terms of human health is very low, whereas contamination by pathogens (such as *E. coli* O157:H7, *Listeria* or *Salmonella*) and by chemicals is the main source of safety problems. Traceability is another quality attribute with increasing importance. Consumers request more detailed information from "the farm to the fork" in order to be able to choose the best products which suit individual needs and expectations.

To summarise, quality and safety issues are increasingly complex problems to solve. We are now in a situation where nutrition has evolved from the prevention of inadequacy (due to the decline in nutritional deficiency) to the issue of health promotion (decreasing of the risk of diseases such as cancer, vascular diseases or diabetes which are on the rise in our society), while eating remains not only a need but also a pleasure. Therefore, current research should be more interdisciplinary (including social, economical and nutritional sciences) to meet the new challenges of society. Important points to consider are the amount of food eaten by individuals, the different typologies of consumers and the dietary diversity associated with a sound way of life, which form the basis for healthiness.

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MAIN PAPER

Effect of different dietary fumonisin B_1 exposure on the toxin content of porcine tissues

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Paper received April 13, 2005; accepted June 24, 2005

ABSTRACT

The time and dose-dependent effect of fumonisin B_1 (FB_1) exposure on the tissue toxin concentrations was examined in 36 weaned pigs. Treated animals were fed an experimental diet supplemented with *Fusarium verticillioides* culture, leading to FB_1 daily intake values of 50 mg/animal (n=10) in a 22 day treatment (T50), 100 mg/animal (n=7) for 5 days ($T100_s$, short exposure) and 100 mg/animal (n=7) for 10 days ($T100_l$, long exposure), respectively. For each experimental group, 4 control (C) animals were used. At the end of trial, the FB_1 content of lung, liver, bile, kidney, brain, spleen, pancreas, heart, muscle and fat samples was determined by LC-MS. Pulmonary oedema developed in all piglets as a result of the toxin dose applied. Highest FB_1 concentrations were found in the liver, kidney, lung and spleen, in all treatments. The muscle and adipose tissues (i.e. the meat) did not contain considerable amounts of fumonisin B_1. In the organs of the animals fed 100 mg FB_1 per day, significantly higher FB_1 levels were measured. An exponential function was descriptive for the measured FB_1 data of some organs. The FB_1 content was found to be dependent on the average daily intake, except in case of the liver.

Key words: Fumonisin B_1, Pig, Mycotoxicosis, Feed contaminant

RIASSUNTO

AUTOMAZIONE DELLA MUNGITURA DELLE BOVINE DA LATTE: RISULTATI SPERIMENTALI E CONSIDERAZIONI

L'effetto della dose e del tempo di esposizione a fumonisina B_1 (FB_1) è stata valutata su 36 lattonzoli sottoposti a concentrazioni tossiche di micotossina. I suinetti sono stati divisi in 4 gruppi sperimentali di cui quello di controllo (n= 12 lattonzoli) era alimentato con la dieta di base e quelli trattati ricevevano la stessa razione integrata con livelli crescenti di una coltura di Fusarium verticillioides *che consentiva l'assunzione di: 50 mg/lattonzolo/giorno (n=10) di FB_1 per un periodo di 22 giorni (trattamento T50); 100 mg/lattonzolo/giorno (n=7) per 5 giorni (trattamento $T100_s$, esposizione breve) e 100 mg/lattonzolo/giorno (n=7) per 10 giorni (trattamento $T100_l$, esposizione prolungata). Al termine della prova campioni di polmone, fegato, bile, reni, cervello, milza, pancreas, cuore, tessuto muscolare e grasso sono stati analizzati per il contenuto di FB_1 con l'ausilio di LC-MS. Tutti i suinetti trattati con FB_1 hanno sviluppato edema polmonare come conseguenza della dose tossica impiegata. In tutti i soggetti trattati, gli organi che hanno evidenziato le maggiori concentrazioni di micotossina sono state il fegato, i reni e i polmoni mentre i tessuti muscolari e adiposi non hanno evidenziato rilevati contaminazioni da fumonisina B_1. Gli organi dei lattonzoli alimentati con 100 mg di FB_1 per giorno hanno evidenziato le concentrazioni significativamente maggiori di micotossina. All'aumentare della dose assunta con la dieta, il contenuto di FB_1 di alcuni organi ha avuto un andamento di tipo esponenziale. La concentrazione di questa mico-*

tossina nei tessuti e negli organi è risultata correlata all'ingestione alimentare gionaliera con unica eccezione per il fegato.

Parole chiave: *Fumonisina B_1, Suino, Micotossine, Contaminanti alimentari.*

Introduction

Fumonisins were first isolated in 1988 from a *Fusarium moniliforme* (*F. verticillioides*) maize-grown culture of the strain MRC 826 (Gelderblom *et al.*, 1988; Cawood *et al.*, 1991), and their molecular structure was also determined (Bezuidenhout *et al.*, 1988). From the viewpoint of toxin exposure, special attention should be paid to fumonisin B_1 (FB_1), being responsible for the development of oesophageal cancer (Marasas *et al.*, 1988) in humans, *leukoencephalomalacia* in horses (Marasas *et al.*, 1988) pulmonary *oedema* in pigs (Fazekas, 1998), hepatic cancer in rats and nephrotoxic and neurotoxic effects in several animal species. Therefore after aflatoxins and ochratoxin, fumonisins are regarded as potentially the most dangerous mycotoxins, mainly with respect to carcinogenic character in humans (IARC, 1993).

The chemical structure of fumonisins is highly similar to that of sphingolipids, therefore they inhibit the biosynthesis of sphingolipids by blocking the sphinganine-N-acyltransferase enzyme action. Alterations induced in the cellular function and morphology can be attributed on one hand to the lack of sphingolipids, and on the other to the accumulation of cytotoxic metabolites such as sphinganine (Kim *et al.*, 1991).

The primary source of FB_1 toxicosis is fumonsin infected maize. The analysis of numerous further food items and raw materials in several countries has revealed that those, listed below, do not mean any health risk from the viewpoint of toxin exposure. Thus, the toxin could be detected from beef (2070 ng/g FB_1 in the liver, 97.3 ng/g FB_1 in the muscle, and 23.4 ng/g FB_1 in the kidney) but only after prolonged exposure to extremely high FB (400 ppm FB_1 and 130 ppm FB_2, for 30 days) doses (Smith and Thakur, 1996). In the United States, fungal culture material containing fumonisin B_1 (75 ppm) was mixed into the total diet and fed for 14 days to two midlactation Jersey cows to determine if fumonisins are excreted in milk. Fumonisins were not detected in any of the milk samples by two analytical laboratories using methods with a sensitivity of 5 ng/ml (Richard *et al.*, 1996). After intravenous administration of ^{14}C-FB_1 to pigs, the accumulation of the toxin could not be demonstrated in the pork, while the liver and the kidney (1076 and 486 ng FB_1 and/or metabolites per g tissue, respectively) were found to be sites of accumulation (Prelusky *et al.*, 1994). In laying hens, the toxin does not form significant residue amounts neither in the different tissues nor in the eggs (Vudathala *et al.*, 1994).

In order to determine fumonisin B_1 residue formation in porcine tissues, the short- and long-time effects of FB_1 fed in different doses were examined in weaned pigs.

Material and methods

In the experiment, 36 weaned (24 treated and 12 control) castrated pigs of the same genotype, weighing 13±1.2 kg, were used. Treated animals were divided into three experimental groups: in T50 (n=10) 50 mg FB_1/animal/day for 22 days, in $T100_s$ (n=7) 100 mg FB_1/animal/day for 5 days and in T100l (n=7) 100 mg FB_1/animal/day for 10 days was fed. For each experimental group, 4 control (C) animals were used.

The experimental animals were fed a basal ration of a composition corresponding to their age (187 g/kg crude protein, 12.8 MJ/kg metabolisable energy, 13.1 g/kg lysine). After a 5-day adaptation period, the *Fusarium verticillioides* fungal culture containing a known amount of FB_1 was added to the treated animals' diet. The fungal culture was produced at the Veterinary Institute of Debrecen according to the method of Fazekas (1998). Control animals were fed a toxin-free diet (T-2, zearalenone, deoxynivalenol and ochratoxin A toxins were not detectable in the diet). The mycotoxin content of the diet was determined by HPLC-system using fluorescence detection, according to the method of Fazekas *et al.* (1996).

All animals were kept individually during the trial. Pigs were weighed weekly in T50 and on the

1st, 5th and 11th day of the experiment in $T100_s$ and $T100_l$. Their clinical status was continuously monitored. Feed was given twice a day, in two equal portions, and the amount of refusals was measured back. Drinking water was available *ad libitum* via automatic drinkers.

One animal was discarded from group $T100_s$ due to insufficient health status that was, however, independent from the toxin exposure.

At the end of the 5, 10 and 22 day-trials pigs were exsanguinated (i.e. on the 6th, 11th and 23th day) after tranquillisation (Vetranquil 1% inj., Phylaxia-Sanofi, Budapest, Hungary) and necropsied.

Gross pathological examination was performed and the lungs, heart, liver, kidneys, spleen, brain and pancreas were weighed. Lung, liver, bile, kidney, brain, spleen, pancreas, heart, muscle (*Longissimus dorsi, Biceps femoris, Psoas major*), subcutaneous and abdominal fat were sampled for FB_1 measurement. The analysis was performed at Institute of Animal Hygiene of the Technical University of Munich, by the HPLC method (Waters 2690 Separations Module, Milford, MA) - MS (VG Platform 2) of Meyer *et al.* (2003).

Results and discussion

In case of 50 mg/animal/day FB_1 dose (T50) no clinical disease signs were found on the pigs. The feed consumption was balanced throughout the experiment and no feed refusal (indicative of toxin effect) was observed. In contrast, animals in groups $T100_s$ and $T100_l$ became depressed, lost appetite, and their feed intake decreased on the 5th-6th day. Pigs showed severe *dyspnoea* while the mucous membranes indicated signs of cyanosis. Clinical symptoms rapidly developed and pulmonary *oedema* led to death within 12-24 hours after the first signs. One and two pigs of $T100_l$ group died on the 5th and 6th day of the experiment, respectively. Two more animals of $T100_l$ group died on the 8th day.

Pulmonary *oedema*, the typical disease entity caused by FB_1 toxin, developed in all piglets, as a result of the doses applied. The thoracic cavity of the pigs contained less or higher amount (15-390 ml) of yellow exudates inclined to coagulation, but by animals in $T100_s$ and T50 groups, the presence of the fluid was observed in more cases and in higher amounts. The liver, the heart and the kidneys showed pathological alterations. The most remarkable pathological findings included e.g. enlarged, friable, pale, yellowish liver, pale kidney with spot-like necrotic areas, and small volume of pericardial fluid, containing lumps. The *trachea* and the *bronchi* contained a white and frothy substance. Haemorrhagic infiltration of the peribronchial lymph nodes was also found, but only in the most serious cases ($T100_s$). Significant difference was not found between the experimental groups and the control in organ weights.

The average total FB_1 intake in $T100_s$ group was 403.8 mg, i.e. 33.1 ± 3 mg/kg BW (Table 1). During the 22-day feeding period (T50), the mean toxin intake of the animals was 1091.2 mg, while the total toxin uptake was 54.6 ± 3 mg/kg of BW. The highest FB_1 concentrations were found in the liver and in the kidneys (Figure 1). Substantial toxin amounts were measured in the myocardium and spleen, while minimal FB_1 concentrations were measured in the lungs.

The total FB_1 intake of the two surviving animals in the $T100_l$ group was on average 742 mg (53 mg/kg BW) in the whole experiment. In both groups ($T100_s$ and $T100_l$), the toxin concentrations of the organs showed very large variations. Particularly high levels were measured in the kidney, the liver, the lung and the spleen (Figure 1). Muscle and fat samples showed negligible contamination (59.8 ±5.6 and 37.4 ±4.8 ng/g, respectively).

These findings show good agreement with the results obtained in the experiment of Prelusky *et al.* (1996), according to which the highest toxin levels could be measured in the liver and kidneys of pigs fed a diet containing 2–3 ppm radiolabelled FB_1 for a period of 24 days.

Individual differences of the toxin content in the organs could be attributed to numerous conditions. On one hand, the cumulative uptake of FB_1 varied between the animals. Due to the rapid clearance of FB_1 in pigs (Prelusky *et al.* 1996), the animals with a daily dose of total appointed FB_1 till death had explicitely higher residues in the tissues than the pigs consuming no or little mycotoxin in the day before exitus. Thus, it is more likely that the tissue concentration is connected with the

Table 1. Calculated parameters of the toxin consumption.

		T50	$T100_s$	$T100_l$
Duration of treatment	d	22	5	10
Animals	n.	10	6	2
Average live weight at the end of trial	kg	20.0	12.2	14.0
Total intake/animal	mg	1091.2	403.8	742
Total intake/body weight	mg/kg BW	54.6	33.1	53
Average daily intake/animal	mg/d	49.6	80.8	74.2
Average daily intake/body weight	mg/kg BW	2.5	6.6	5.3

mycotoxin uptake directly prior to the death. Variations in the individual absorption efficacy and metabolism may also have contributed to the differences of detectable residues.

The liver, kidney, spleen and lung were the organs with the highest toxin contents; according to the FB_1 intake, significant differences ($P \leq 0.05$) were found among them in groups $T100_s$, $T100_l$ and T50 (Figure 1). Obviously, significantly higher levels were measured in the organs of the group $T100_s$.

No detectable levels of fumonisin B_1 were measured in the organs of the control pigs.

Comparing data of table 1. and figure 1., a negative correlation was supposed between the absolute toxin intake and the toxin content of the organs, i.e. the more toxin got into the organism the less was accumulated in the organs. However, it must be noted that in $T100_s$ and $T100_l$ groups the toxin was consumed only for a short period, accordingly, the average daily intake per animal and per body weight was higher than in T50, while the total consumed FB_1 amount was less. Accordingly, from the viewpoint of toxin exposure not the absolute intake during a period is determinant, but the daily toxin amount, what is incorporated by the organism directly before the slaughter. This is supported by the close positive correlations found between the average daily intake (Table 1) and the toxin content of some organs (Table 2).

Figure 1. The average FB_1 concentration of certain organs related to daily intake (dark columns are averages of $T100_s$ and $T100_l$ groups) (*$P \leq 0.05$).

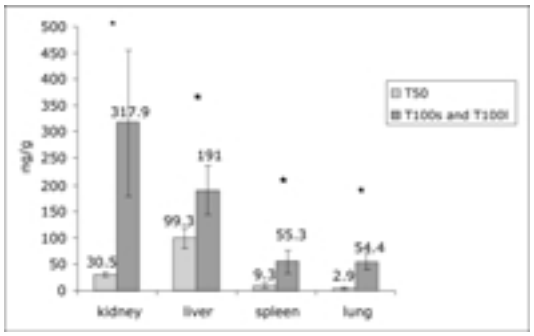
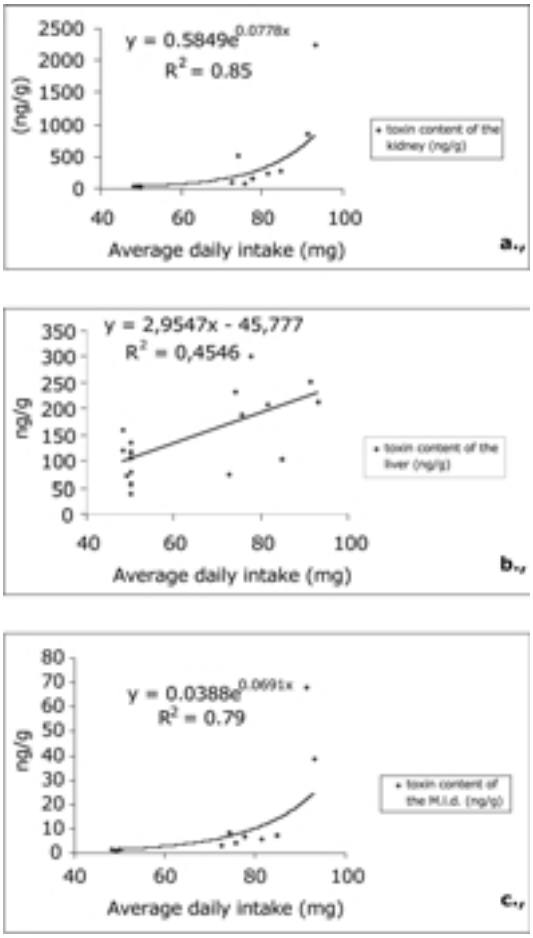


Figure 2. shows the relationship between the average daily toxin intake and toxin- concentration of the kidney, the liver and the muscle *Longissimus dorsi*. In case of the kidney and the muscle, an exponential curve was fit to the data. Close positive correlations among variable-pairs, moreover, a relatively well-adapting exponential

Table 2. Correlations between the average daily FB1 intake and the toxin content of some organs.

Organ	No. of animals with detectable toxin concentration	Correlation (r)	Significance (P)
Liver	18	0.674	0.002
Kidney	18	0.671	0.002
Longissimus dorsi	11	0.632	0.037

Figure 2. Relationship between the average daily FB_1 intake and some tissues' (kidney (a), liver (b) and Longissimus dorsi (c)) toxin concentration.



function was found, with high "R^2" values. For the liver – presumably due to a relative high standard deviation (mean: 142.5 ng/g; S.D.: 74.6) – a linear relationship (Figure 2b) could be better adapted.

Conclusions

In all groups the highest concentrations of FB_1 were found in the liver, kidney, spleen and lung. According to the FB_1 intake, significant differences ($P \leq 0.05$) were found among them in groups T50, $T100_s$ and $T100_l$. The muscle and adipose tissue did not contain considerable amounts of fumonisin B_1. An exponential function may adapt to data of some organs' FB_1 content being dependent on the average daily intake, except the liver (linear relationship). Because the toxin does not cumulate in the organism, not the absolute toxin amount consumed, but rather the daily intake seems to be determinant from the viewpoint of toxin exposure.

This research was supported by the Hungarian Academy of Sciences (project no. B04074) and the Ministry of Education (NKFP 4/034/2001).

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SHORT COMMUNICATION

Differentiation of pork *longissimus dorsi* muscle regarding the variation in water holding capacity and correlated traits

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ABSTRACT

This study was performed on 87 randomly selected carcasses of castrated pigs, slaughtered at approximately 100 kg live weight in 2 abattoirs from east Croatia. Important meat quality traits were measured on the samples of *musculus longissimus dorsi*: initial and ultimate pH values (pH_{45} and pH_{24}), meat colour and drip loss. Samples were further differentiated into 5 groups on the base of meat quality conditions. Results show that 14.66%, 32%, 40%, 10.67% and 2.66% of the *longissimus dorsi* muscles belonged to PSE, RSE, RFN, PFN and DFD conditions respectively. The highest correlations were observed between ultimate pH value, drip loss and meat colour. Interpolated threshold value for ultimate pH was 5.69. It is suggested to verify the values used for differentiation of meat according to quality on a larger sample.

Key words: Pork, *Longissimus dorsi* muscle, Meat quality traits, Differentiation

Introduction

The quality of pork is a constant issue which occupies the mind of many scientists for a long time. Together with the development of different methods aimed at measuring and quantifying the pork quality, various systems for differentiation of pork according to quality evolved. These differentiations are mainly based upon the measurements of pH decline rate, ultimate pH values, water holding capacity, muscle colour, firmness, marbling etc. Pork is often differentiated into quality conditions such as: pale, soft, exudative (PSE); reddish-pink, firm, nonexudative (RFN or normal) and dark, firm, dry (DFD) pork. Sellier and Monin (1994) described PSE meat having pH_{45} less than 5.8; pH_{24} less than 5.5, drip loss more than 5% and colour - CIE L* more than 50. Other authors used different criteria. For example, Kauffman *et al.* (1992) and Warner *et al.* (1997) used drip loss >5% to differentiate PSE meat; Joo *et al.* (1999) set up drip loss threshold values for PSE meat to be >6%. Forrest (1998) reported that when pH_{24} is 5.5 or lower, nearly 99% of the pork is PSE; while van Laack (2000) used pH_{24} less than 5.7 as the indicator of PSE meat. Kauffman *et al.* (1992) suggested two additional quality conditions: RSE (reddish-pink, soft, exudative) and PFN (pale, firm, non-exudative) with their own threshold values of measured indicators. The aim of the present paper is to examine the relation between important meat quality traits and to determine certain threshold values in order to differentiate investigated pork *longissimus dorsi* muscles according to quality conditions.

Material and methods

This study was performed on 87 randomly selected carcasses of barrows, slaughtered at approximately 100 kg live weight in two abattoirs from east Croatia. At the slaughter line, 45 minutes after sticking, initial pH values were taken at the *longissimus dorsi* muscle of primarily processed swine carcasses. After 24 hours of cooling, ultimate pH values and colour of *m. longissimus dorsi* were measured. Water holding capacity was expressed as drip loss and determined by the method described by Kauffman *et al.* (1992); the colour of meat was measured by "Minolta CR-300" device at *m. longissimus dorsi* cut after 15 minutes of blooming and presented as CIE L* values. The measurements of pH_{45} and pH_{24} were carried out by digital pH-meter "Mettler MP 120-B". Statistical analysis was performed using STATISTICA (6.0) for Windows program. Descriptive statistics and multiple correlation analysis procedures were used. Threshold value for ultimate pH was calculated using linear equation y=a+bx, where y=drip loss, a=intercept, b=slope and x=pH_{24}. Figure was produced using Microsoft Excel 2002.

Results and conclusions

Meat quality traits of investigated pigs are presented in table 1. Although the mean values of initial and ultimate pH values respected the reference values for "normal" meat, it is obvious that drip loss is to some extent higher than proposed by some authors (Sellier and Monin, 1994; Kauffman *et al.*, 1992; Warner *et al.*, 1997).

Better view at the meat quality of investigated *longissimus dorsi* muscles was obtained when samples were distributed into meat quality conditions as presented on figure 1. This distribution shows that 14.66% and 32% of the samples expressed PSE and RSE condition, respectively. These groups are characterized by ultimate pH values less than 6, excessive drip loss (>5%) and CIE-L* values higher than 50 in case of PSE and 42-50 for RSE meat. RFN meat (called "normal") was represented with 40% of the muscle *longissimus dorsi* samples (pH_{24}>6; drip loss <5% and CIE-L* 42-50). PFN condition of the meat can be viewed as meat with paler colour, but other traits quite desirable (pH_{24}>6; drip loss <5%; CIE-L* >50). To these condition 10.67% of the samples were classified. Only 2.66% of the pork was distributed into DFD condition of the meat (pH_{24}>6; drip loss <5% CIE-L*<42). For the meat processing industry this classification is more of use than the differentiation of meat on the base of only one parameter, because it gives the basis for better decisions about the further purpose of examined pork muscles. However, larger sample should be taken in consideration in order to draw more general conclusions than in this study. A good exam-

Figure 1. Differentiation of pork *longissimus dorsi* muscle into quality conditions according to Kauffman *et al.* (1992).

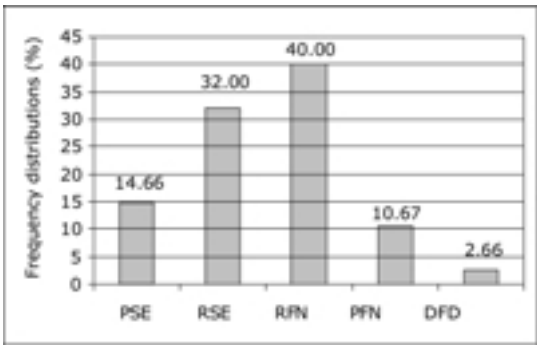

Table 1. Descriptive statistics for meat quality traits of investigated pork samples.

Trait	Mean	Standard Deviation	Min.	Max.
pH_{45}	6.11	0.28	5.43	6.62
pH_{24}	5.63	0.17	5.41	6.46
CIE-L*	46.49	4.09	35.65	55.79
Drip loss (%)	5.23	1.99	1.45	10.18

ple for this is the work of Cassens *et al.* (1992) who found more than 50% of the *m. gluteus medius* to be RSE and only 15% was "normal" (RFN) in the survey of 14 pork processing plants in USA.

The manners used to differentiate pork muscles into quality conditions may vary considerably depending on the methods used, but also on the measuring instruments, pig population (breed, genotype, etc.) slaughter conditions and other factors of influence. Nevertheless, many authors reported ultimate pH values as the most convincing predictor of meat quality expressed as the ability to withhold water which is difficult to measure at the slaughter plants; this is due to high correlation between pH_{24} and WHC (Forrest, 1998; van Laack, 2000). Ultimate pH value used to differentiate PSE meat from other conditions according to those authors varies between 5.5 and 5.7. Correlation coefficients calculated for measured quality traits in this trial are shown in table 2. It is obvious that the highest correlations observed were between ultimate pH value and drip loss as well as CIE-L* values as the measure of brightness (paleness). Similar results are reported by Otto *et al.* (2004), while Eikelenboom *et al.* (1995) and Kralik *et al.* (2002) reported to some extent higher correlation than in current study between pH_{24} and Minolta L* and water holding capacity. Kušec *et al.* (2003) found significant correlation between initial pH, ultimate pH value and WHC in the meat samples from the Duroc sired pig carcasses, while results found for the samples originated from Pietrain sired carcasses resembled those presented here.

The relation between ultimate pH value and drip loss was described by linear equation: Drip loss = $33.6728 - 5.04*pH_{24}$. Interpolated threshold value of ultimate pH was 5.69. Although interpolated ultimate pH was close to the value reported by van Laack (2002) it should be verified on a larger sample in order to draw more general conclusions due to different reports from other authors.

Table 2. Correlation coefficients for meat quality traits of *longissimus dorsi muscle* of investigated pig carcasses (n=87).

Traits	pH_{45}	pH_{24}	Drip loss (%)
pH_{45}	-		
pH_{24}	0.088 n.s.	-	
Drip loss (%)	-0.133 n.s.	-0.406**	-
CIE- L*	-0.031 n.s.	-0.392**	0.384**

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SHORT COMMUNICATION

Relationship between raw ham cathepsin B activity and firmness of dry cured hams

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ABSTRACT

This study aimed to investigate the relationship between cathepsin B activity and muscle firmness of dry cured hams. A total of 988 samples of semimembranosus muscle were collected from raw hams of heavy pigs and cathepsin B activity was determined using fluorimetric method. Raw hams were cured following San Daniele guidelines. Dry-cured hams were deboned and cross-sectioned. On the cross section firmness was measured at three muscular sites (*M. semimembranosus, semitendinosus* and *biceps femoris*) using a Hardness Meter MK2. This study did not evidence any significant relationship between cathepsin activity and firmness of dry cured hams.

Key words: Pigs, Dry cured ham, Cathepsin B activity, Firmness

Introduction

Dry-cured ham is a typical Italian meat product of high commercial value. The processing of dry-cured hams involves a long maturation period where proteolysis and lipolysis reactions take place (Toldrà *et al.*, 1992). Proteins breakdown mechanisms mostly depend on muscle lysosomial proteinases (Parolari *et al.*, 1994). Cathepsins are lysosomial proteinases that have been associated to intense protein cleavage occurring throughout ripening (Toldrà and Etherington, 1988; Toldrà *et al.*, 1992). These enzymes are active at acid pH and are able to degrade myofibrillar proteins. Cathepsins maintain 40-50% of initial activity after 8 months of curing (Toldrà and Etherington, 1988) and play an important role in biochemical processes because in dry-cured hams proteolytic activity is controlled by muscular enzymes with no microbial intervention. Some studies showed that, in raw hams, cathepsin B residual activity, proteolysis index and texture are highly correlated traits (Virgili *et al.*, 1994). However, relationships between initial activity and quality traits of end products have been scarcely investigated. This study aimed to investigate the relationship between enzymatic activity of cathepsin B in raw hams and firmness of dry cured hams.

Material and methods

This study used data from 988 (494 castrated males and 494 gilts) crossbred heavy pigs slaughtered at the same abattoir. Pigs were progeny of 49 Gorzagri C21 Large White boars and 141 crossbred Large White-derived sows. Hams were dressed after 24 h of refrigeration, pH was measured at dressing on left thighs, and samples of semimembranosus muscle were collected from all left hams for cathepsin B activity determination. Cathepsin B activity was determined two days

Table 1. Descriptive statistics of carcass weight and ham traits.

Variable	Mean	Standard Deviation	C. V. (%)
Carcass weight (kg)	136.6	13.6	9.9
pH after 45 min	6.33	0.19	3.0
pH after 24 hours	5.77	0.16	2.8
Cathepsin B activity (nmol AMC min^{-1} g^{-1})	1.36	0.31	22.8
Firmness of muscles:			
- Biceps femoris	563	80.4	14.3
- Semimembranosus	738	80.2	10.9
- Semitendinosus	586	87.8	15.0

after slaughtering using analytical procedures described by Schivazappa *et al.*, (1992) and Sturaro *et al.* (2004). Raw hams were cured following San Daniele guidelines (1996). At the end of curing, hams were deboned and cross-sectioned. Firmness of the lean fraction was measured on biceps femoris, semitendinosus and semimembranosus muscles using a Hardness Meter MK2 (Noventa *et al.*, 2004).

Statistical analysis

Measures of cathepsin activity were analysed by ANOVA (SAS user's guide, 1990) using a linear model which included the fixed effects of the slaughter group (24 groups), sex (castrated males and females), carcass weight (5 classes) and the effect of pH measured at 24 h after slaughtering (covariable). To investigate the influence of enzymatic activity on muscle firmness, data collected by Hardness Meter were analysed by ANOVA using a linear model which included the fixed effects of the slaughter group, sex, carcass weight, and cathepsin B activity (covariable).

Results and conclusions

Descriptive statistics of carcass weight and ham traits are reported in Table 1. Average carcass weight was 137 kg with moderate value of coefficient of variation. Values of pH at 45 min and 24 h after slaughtering showed limited variability and confirmed absence of PSE and DFD in the sample analysed. Cathepsin B activity exhibited an average value of 1.36 nmol AMC min^{-1} g^{-1} and a large variability (C.V. = 22.8 %).

Muscle firmness was higher in semimembranosus than in other muscular sites, and not much different between semitendinosus and biceps femoris muscles. Coefficients of variability for muscles firmness ranged from 11 to 15%. Correlations for firmness of different muscles and between cathepsin activity and firmness are reported in Table 2. Coefficients indicate a high correlation between firmness of biceps femoris and that of semitendinosus, and a moderate correlation between semimembranosus and the other muscles. Relationships between cathepsin activity and firmness were low. There was a not significant correlation between cathepsin B activity and semitendinosus firmness whereas correlations between enzymatic activity and firmness of the other muscles, albeit being significant, were small.

Results of ANOVA for muscles firmness and enzymatic activity are reported in Table 3. All

Table 2. Correlations (%) for firmness of different muscles and between firmness and cathepsin B.

	Biceps femoris	Semitendinosus	Semimembranosus
Cathepsin B activity	10.4 *	5.8 n.s.	20.0 ***
Biceps femoris		70.0 ***	54.8 ***
Semitendinosus			58.3 ***

**** P < .001, * P < 0.05, n.s.= not significant*

Table 3. ANOVA for muscles firmness and cathepsin B activity.

Effect	F statistics			
	Cathepsin B activity	Biceps femoris	Semi-tendinosus	Semi-membranosus
Slaughter group	35.4***	9.4***	10.8***	14.0***
Sex	5.4*	n.s.	11.2**	n.s.
Carcass weight	2.1*	21.1***	16.6***	9.6***
pH at 24 h after slaughtering	13.6***	-	-	-
Cathepsin B activity (nmol AMC min^{-1} g^{-1})	-	n.s.	n.s.	n.s.
R^2 (%)	47.5	27.9	28.8	29.9

**** P < 0.001, ** P < 0.01, * P < 0.05, n.s.= not significant*

effects included in the linear model significantly affected cathepsin activity. Effects due to the slaughter group and pH at 24 h after slaughtering were the most important sources of variation for cathepsin B activity. Coefficient of determination of the model for cathepsin activity was 47.5%. Values of R^2 for firmness were lower ranging from 27.9% (biceps femoris) to 29.9% (semimembranous). Effects due to the slaughter group and carcass weight were significant sources of variation for all muscular sites. Sex influenced firmness of semitendinosus muscle only ($P < 0.01$). Muscles firmness tended to decrease at increasing carcass weight (data not reported in table). Cathepsin B activity was not a significant source of variation for muscles firmness. Parolari *et al.* (1994) and Schivazappa *et al.* (2002) reported significant relationships between cathepsin B activity and intensity of proteolysis during curing and Virgili *et al.* (1995) postulated a significant relationship between firmness of dry cured hams and enzymatic activity. However, those studies analysed samples of limited size. In conclusion, cathepsin B activity measured in the fresh ham does not seem to be a relevant trait in relation to firmness characteristics of dry cured hams.

Financial support by Montorsi S.p.A. is gratefully acknowledged.

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SHORT COMMUNICATION

Influence of ham weight, trimming and pressing on Istrian dry-cured ham seasoning loss

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ABSTRACT

Influence of raw ham weight, trimming form and pressing on seasoning weight loss of Istrian dry-cured hams were studied. Swine thighs were shaped traditionally with skin and subcutaneous fat removed. The pelvis bones were left in the ham (B hams) or experimentally deboned (boneless or BL hams). After trimming the raw hams were weighted and classified into the weight classes. The hams were pressed after dry salting with different loads. High seasoning weight loss (46.31 %) of Istrian hams was expected due to raw ham skinning. However, deboning had no influence on differences in seasoning weight loss. Significantly higher weight loss of light class hams suggest to use raw hams heavier than 10 kg and 9 kg after B and BL dressing, respectively. There was no significant press load effect on seasoning weight loss.

Key words: Istrian dry-cured ham, Trimming, Pressing, Seasoning weight loss

Introduction

The seasoning weight loss in the production of dry cured ham measure the extent of meat dehydration, expressed as percentage ratio between weight loss at the end of different stage of seasoning and weight of trimmed ham at the start of the process. It may vary from 20 % to over 30 % and could cause significant implications on the production yield depending on the production technology, ham weight, lean meat content, adipose covering of fresh ham, the age and breed of pigs (see review of Russo and Nanni Costa, 1995). Traditionally, the Istrian dry-cured ham is made of raw ham trimmed uniquely with intact pelvis bones left in the ham and with skin and subcutaneous fat removed. This practice was maintained from old times when the requirements for grease were of high importance. Due to skin and fat removal higher seasoning weight loss of Istrian ham is expected (Puljić, 1986). Recently, more raw hams are trimmed without pelvis bones due to increasing market demands for easy sliceable product. However, as deboning causes breaking of anatomical integrity of muscles it may lead to increasing weight loss of ham due to larger meat surface exposure to evaporation. This study was designed to test the effect of ham trimming (with or without pelvis bones) and ham weight on seasoning weight loss of Istrian ham. Additionally, the influence of three different press weights on seasoning weight loss was investigated.

Material and methods

Raw material and ham processing: one hundred hams originated from Swedish Landrace pigs

reared at the same farm with similar conditions of housing and feeding were selected for dry-cured ham processing. Live weights of pigs at the time of slaughter were between 100 and 120 kg. All hams were cut from the carcass between last lumbar vertebra (*vertebrae lumbales*) and sacrum with foot removed between tarsal bones and tibia. Raw hams were then entirely skinned with subcutaneous fat removed except for 10 cm wide zone near the distal cut. Half of the hams were randomly selected and trimmed into a characteristic traditional shape by removing only the sacrum and tail bones (*vertebrae caudalis*) with pelvis bones (*os ilium, os ischii* and *os pubis*) left in the ham (B hams). The rest 50 hams were trimmed without pelvis bones which were cut between head of the femur and cup-shaped acetabulum of the hip bone (boneless or BL hams). The caudal part of the aitchbone (*tuber ischii*) was left in the ham. In addition, the hip musculature was semi-circularly shaped by knife. After trimming the individual weights of all hams were recorded. The hams were classified into the following weight classes for B hams: I>12 kg, 10<II≤12 kg and III≤10 kg; for BL hams: I>11 kg, 9<II≤11 kg and III≤9 kg. Both B and BL hams were cured according to typical Istrian dry-curing procedure which takes place seasonally during cold part of the year. The hams were first thoroughly rubbed with dry sea salt, piled and left in a cold room for two weeks. Then they were pressed for another week and washed for 24h to eliminate the excess salt. During pressing, the hams were set in the four rows between boards and experimentally, three different pressures were loaded at the uppermost board: 150, 200 and 250 kgm^{-2} for 25, 50 and 25 hams, respectively. The hams were randomly assigned to the different pressure loads. After salting and pressing, all hams were hanged for three months in a drying room exposed to windy Istrian climate. After drying the hams were kept in a cool and dark cellar until the attainment of commercial maturity at the age of 12 months. Seasoning weight loss measurement: the hams were weighted individually at the start of process (after trimming), after three weeks (at the end of the salting/pressing), after 4 months (at the end of drying), after 6 months (during ripening), and after one year (at the commercial maturity stage). Seasoning weight loss (%) was calculated with formulae:

$$\text{Seasoning weight loss (\%)} = \frac{\text{Ham weigth at start (kg)} - \text{Ham weigth during process (kg)}}{\text{Ham weigth at start (kg)}} \times 100$$

Statistical Analysis: the seasoning weight loss records were treated as repeated measures and examined with analysis of variance using the MIXED procedure (SAS, 1999). Ham weight class, trimming form and pressing weight and their interactions with weighing time were defined as fixed while the ham was defined as a random effect. Means were separated and standard errors were calculated using the least squares means statement (LSMEANS) with PDIFF and STDERR options, respectively. The Tukey-Kramer test was applied to compare the means at the level of significance at $P<0.05$ and $P<0.01$.

Results and conclusions

The results of seasoning weight loss of Istrian dry-cured hams are shown in Table 1. The average seasoning weight loss of mature Istrian dry-cured hams was 46.31 % what is considerably higher than in Dalmatian (37.7 %; Puljić, 1986), Bayonne (from 35 to 39 %; Monin *et al.*, 1997), Parma (about 27 %; Nanni Costa *et al.*, 1999) or Serrano dry-cured ham (from 34 to 35 %; Gou *et al.*, 1995). During the processing the weight losses of BL hams were slightly higher than in B hams, but the statistically significant difference ($P<0.05$) was found only after drying stage. No statistically significant difference in seasoning weight loss was observed between the hams in weight classes I and II. However, the weight loss of light hams (III class) was highest in all processing stages and at the end of the process it was 48.11 % what is for 2.88 % ($P<0.05$) and 2.53 % ($P<0.01$) higher than in I and II class hams, respectively. Although the hams which were pressed under 250 kg had significantly higher ($P<0.01$) weight lost immediately after pressing the differences disappeared at the end of the process. Results of this experiment confirm high seasoning weight loss of Istrian dry-cured ham due to raw ham skin and fat removal,

Table 1. Processing stages and seasoning weight loss (%) of Istrian dry-cured hams.

	Processing stages							
	Salting/pressing 3 weeks		Drying 4 months		Ripening 6 months		Commercial maturity 12 months	
	n%	LSM±SE	n%	LSM±SE	n%	LSM±SE	n%	LSM±SE
All samples	100	5.79±0.28	100	30.37±0.28	96	38.52±0.28	89	46.30±0.29
Dressing form[1]								
B	50	5.49±0.22	50	29.24[a]±0.43	49	37.60±0.50	44	46.20±0.45
BL	50	5.92±0.22	50	31.33[b]±0.43	47	39.53±0.50	45	46.40±0.45
Weight class[2]								
I	22	4.80±0.56	22	28.66[A]±0.56	19	36.18[A]±0.61	16	45.23[a]±0.66
II	48	5.66±0.38	48	29.75[A]±0.38	48	37.79[A]±0.38	44	45.58[A]±0.40
III	30	6.45±0.48	30	32.35[B]±0.48	29	40.76[B]±0.49	29	48.11[B]±0.49
Pressing weight[3]								
150	25	5.51[A]±0.25	25	29.76±0.62	25	38.28±0.72	21	46.25±0.63
200	50	5.04[A]±0.18	50	29.71±0.44	48	38.10±0.51	46	46.16±0.44
250	25	7.22[B]±0.25	25	31.95±0.62	23	39.69±0.72	22	46.35±0.63

n - number of hams , LSM±SE - least square means±standard error

[1]B - hams with pelvic bones, BL - hams without pelvic bones.

[2]after dressing, for B hams: I>12 kg, 10<II ! 12 kg and III ! 10 kg; for BL hams: I>11 kg , 9<II ! 11 and III ! 9kg.

[3]kgm[-2]

Means with different superscripts within column for [1,2,3] differ significantly: P<0.05=a,b; P<0.01=A,B

whereas deboning of pelvis bones had no significant influence on seasoning weight loss. Significantly higher seasoning weight loss of light hams suggest to use raw hams heavier than 10 kg and 9 kg after B and BL dressing, respectively. There was no significant press load effect on seasoning weight loss.

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SHORT COMMUNICATION

Dietary supplementation with olive stone meal in growing rabbits

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ABSTRACT

Olive stone meal is a low-digested fibre source potentially useful in the prevention of digestive troubles in growing rabbit permitting a better balance of dietary fibre fractions. To evaluate its efficacy, three experimental diets containing 0, 3 or 6% olive stone meal were fed to 222 rabbits from weaning (28 d) to slaughter (73 d). Olive stone inclusion increased the proportion of large dietary particles while did not affect growth performance, digestive physiology and carcass and meat quality. Due to optimum health status observed in all experimental groups, the preventive action of olive stone meal against the occurrence of digestive troubles was not proven.

Key words: Rabbits, Olive stone meal, Digestive physiology, Growth performance

Introduction

Digestive troubles of multifactorial ethiology are the first cause of mortality in growing rabbits (Licois, 2004). Besides to characterize the implicated pathogenic agents, it is fundamental to individuate and eliminate the environmental and dietary factors favouring the disease outbreak. Adequate levels of indigestible fibre fractions (ADF>20% DM, ADL>5.5% DM) are recommended to improve the digestive health of weaning and growing rabbits (Gidenne, 2003). These concentrations are not easy to be reached in practical formulation without reducing the nutritive value of compound feeds. The dietary inclusion of olive stone meal as a source of indigestible fibre could permit a better equilibrium among the different fibre fractions therefore reducing sanitary risk without impairing growth performance and feed conversion.

Material and methods

At 28 d, 222 rabbits (live weight 676 ± 49 g) were weaned, assigned to three groups of 74 animals and fed *ad libitum* the experimental diets B0, B3 and B6 containing respectively 0, 3 and 6% of olive stone meal. This feedstuff was grounded roughly and contained (DM basis) CP 5.0%, NDF 78.4%, ADF 64.0% and ADL 33.1%. The compound diets were formulated to have similar digestible protein (DP) to digestible energy (DE) ratio and similar digestible fibre (hemicelluloses and pectins) and ADL concentrations (Table 1). No antibiotics or additives were administered either in feed or water along the trial. At 49 and 66 d, 60 rabbits (10 per diet and age) were sacrificed to sample ileal mucosa and collect caecal content. At 73 d, 120 rabbits (40 per diet) were slaughtered, carcasses were dissected and meat quality was evaluated following the methods described by Xiccato *et al.* (1994). *In vivo* nutrient digestibility and chemical composition of diets, faeces and cae-

Table 1. Main ingredients and chemical, physical and nutritive characteristics of experimental diets.

	Diet B0	Diet B3	Diet B6
Main ingredients (%):			
Olive stone meal	0	3	6
Dehydrated alfalfa meal, CP 17%	38	33	28
Barley (six rows)	15	15	15
Wheat bran and middlings	25	26	27
Beet pulp	6	8	10
Soybean meal, CP 44%	0	5	10
Sunflower meal, CP 30%	12	6	0
Chemical composition (% DM):			
Dry matter	90.2	90.3	90.3
Crude protein	17.2	17.5	18.0
NDF	42.1	40.2	39.2
ADF	22.5	21.3	19.5
ADL	5.9	5.8	5.7
Digestible fibre (hemic.+pectins)	20.9	21.0	20.6
Starch	18.6	19.5	20.4
Digestibility and nutritive value:			
DM digestibility, %	59.6	61.2	63.9
DE, MJ/kg DM	11.2	11.1	11.6
DP to DE ratio, g/MJ	11.7	11.7	11.8
Particle size distribution (%):			
> 1.18 mm	6.8	9.0	11.0
0.60 to 1.18 mm	14.5	13.6	13.0
0.30 to 0.60 mm	12.4	11.3	10.6
< 0.30 mm	66.3	66.1	65.4

cal content were determined as detailed by Xiccato *et al.* (2003). Diet particle size distribution was measured in triplicate according to García *et al.* (2000). Villi height and cripth depth were measured on samples of ileal mucosa. Data were analysed considering diet as the main variability factor and using the GLM procedure of SAS (1991). The effect of age and its interaction with diet were considered when analysing data of caecal fermentation activity and ileal mucosa.

Results and conclusions

Regardless from some differences in protein and fibre fraction concentrations among experimental diets (Table 1), chemical composition and nutritive value were in agreement with the current recommendations for weaning and fattening rabbits (De Blas and Mateos, 1998). Only the starch level was higher than what recommended for young rabbits (<14% DM). DM digestibility raised from diet B0 to B6, due to the higher starch concentration and the higher digestibility of digestible fibre fractions (hemicelluloses and pectins, data not reported) in comparison with diet B0. With the inclusion of olive stone meal, large particle proportion (>1.18 mm) increased, while the finest particles (<0.30 mm), which are preferentially involved in caecotrophy (García *et al.*, 2000), did not vary.

Although the experimental diets contained high starch concentrations potentially favouring digestive diseases in young rabbits, all animals were in good health during the whole experiment apart from 9 rabbits dead or excluded due to respiratory problems. The optimum initial health condition of weaned rabbits and the balanced levels of dietary fibre fractions (Gidenne, 2003) likely accounted for the absence of digestive troubles. The inclusion of olive stone could have further con-

Table 2. Growth performance from 28 to 73 d of age.

	Diet B0	Diet B3	Diet B6	*Prob.*	RSD
Rabbits, no.	49	53	51		
Initial live weight, g	677	673	677	0.90	49
Final live weight, g	2593	2571	2594	0.71	173
Daily weight gain, g/d	42.6	42.2	42.6	0.76	3.5
Daily feed intake, g/d	125^{A}	120B	117^{C}	<0.001	10
Feed conversion	2.95^{A}	2.85B	2.74^{C}	<0.001	0.16

tributed to compensate the supposed negative effect of the higher starch concentration in diets B3 and B6 in comparison with diet B0. Daily weight gain and final live weight were not affected by olive stone meal inclusion, whereas daily feed intake decreased and consequently feed conversion improved (P<0.001) from diet B0 to diet B6 (Table 2).

Caecal fermentation (Table 3) was not modified by the dietary treatment nor at 49 or 63 d of age, with pH, ammonia and total volatile fatty acid (VFA) concentrations typical of healthy rabbits (Gidenne, 2003, Xiccato *et al.*, 2003). Differently, the proportions of acetate decreased and conversely that of butyrate increased in rabbits fed diet B6 in comparison with diets B0 and B3 (P≤0.05), to be ascribed to the higher dietary starch concentration. The morphology of ileal mucosa was not influenced by the dietary treatment. Similarly, carcass traits and meat quality were not affected.

In conclusion, olive stone meal can be included in diets for growing rabbits increasing fibre supply and permitting a suitable balance among more and less digestible fibre fractions. In the present study, the inclusion of roughly-grounded olive stone increased the proportion of large dietary particles, but did not affect growth performance, digestive physiology and meat quality. Further research under unfavourable health conditions is needed to demonstrate the hypothesised positive effect of olive stone in the prevention of digestive diseases.

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Table 3. Caecal fermentation activity and ileal mucosa morphometry (mean of 49 and 63 d values).

	Diet B0	Diet B3	Diet B6	*Prob.*	RSD
Rabbits, no.	49	53	51		
Initial live weight, g	677	673	677	0.90	49
Final live weight, g	2593	2571	2594	0.71	173
Daily weight gain, g/d	42.6	42.2	42.6	0.76	3.5
Daily feed intake, g/d	125^{A}	120B	117^{C}	<0.001	10
Feed conversion	2.95^{A}	2.85B	2.74^{C}	<0.001	0.16

SHORT COMMUNICATION

The influence of different fat sources on fattening of turkeys and composition of fatty acids in breast muscles

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ABSTRACT

The aim of this research was to evaluate the influence that different fat sources (Bergafat-BF, Pronova Biocare Epax 3000 TG-PBE and rape oil-RO) have on fattening characteristics of turkeys and composition of lipids in breast muscles, if added separately in the amount of 3% and also equally combined in finishing diets. The research was carried out on 180 heavy hybrid turkeys of Nicholas 700 provenience. Different sources of fat did not have statistically significant effect ($P>0.05$) on finishing weights, average daily weight gain, consumption and feed conversion. However, supplementation of the above stated fat sources changed highly significantly ($P<0.001$) the content of SFA, MUFA and EPA+DHA in the lipids of breast muscles. Rape oil, which was added to diets, had positive effect on the content of SFA and MUFA. In comparison to non-supplemented diets, supplementation of Pronova preparation to diets resulted in doubling of the desirable EPA+DHA.

Key words: Turkeys, Fattening, Fatty acids, EPA+DHA

Introduction

There has been recently a significant emphasis put on turkey production in developed countries worldwide. Irregular nutrition of humans and their constant exposure to stress may cause some cardiovascular diseases, heart attach or stroke, leading often to death (Barlow and Pike, 1991, Calvani and Benatti, 2003). One of the factors to cause these diseases is the imbalanced ratio of omega n-6:omega n-3 acids. Supplementation of vegetable oils to poultry diets decreases the ratio of omega n-6:omega n-3, mostly through the ratio of linoleic (C18:2n-6) to α-linolenic (C18:3n-3) acid. However, within the group of omega n-3 polyunsaturated fatty acids, greater importance is given to eicosapentaenoic-EPA and docosahexaenoic-DHA acid (Ollis *et al.*, 1999, Simopoulos, 2000, Komprda *et al.*, 2003, Ivanković and Kralik, 2004). Consumption of at least 0.5 g of omega-3 fatty acids a day is proved to have positive effect on human health (Mantzioris, 2000; Simopoulos, 2000). The aim of this research was to determine the influence of different fats and oils on fattening of turkeys and on composition of fatty acids in the lipids of breast muscles.

Material and methods

The research was carried out on 180 turkeys of Nicholas 700 provenience. Turkeys were divided in six groups of 30, and the experiment was conducted in three replications during 15th-19th week of fattening. Diets fed to turkeys differed in supplemented fats, as overviewed in Table 1. During the experiment live weights were controlled, on the

Table 1. Ingredients and calculated composition of diets.

Ingredient, %	Finisher (from week 15 to the end of fattening)	Calculated composition of diets		
Corn	51.44	Crude protein	%	19.50
Extruded soybean	22.41	Fat	%	10.00
Soybean cake, 46%	11.61	Crude fiber	%	3.43
Yeast, 52%	5.00	Ash	%	6.22
Phosphonal	2.62	Lysine	%	1.45
Methionine	0.21	Methionine	%	0.50
Lysine	0.04	Triptophan	%	0.26
Salt	0.20	Arginine	%	1.40
Lignobond (binder)	1.00	Ca	%	1.05
Limestone	0.47	P usable	%	0.55
Premix	1.00	Na	%	0.17
Fat (oil)	3.00	Linoleic acid	%	3.09
Pigosen	1.00	ME	MJ/kg	13.54
TOTAL	100.00			

basis of which the average weekly weight gain was calculated. Feed consumption and conversion into live weight (g/g) were also determined and presented for the whole fattening period. The following fat sources in the amount of 3% were used as supplements in diets (Table 1): Bergafat (BF), Pronova Biocare Epax 3000 TG (PBE) and rape oil (RO). According to producer's declaration, BF preparation contained 30-44% saturated fatty acids (SFA), 35-45% monounsaturated fatty acids (MUFA) and less than 4% arachidic acid (C20:0), as well as other fatty acids with 20 or more carbonic atoms in chain. The PBE preparation contained 27-29% SFA, 24-26% MUFA, 15.36% eicosapentaenoic acid (EPA, C20:5n-3) and 9.99% docosahexaenoic acid (DHA, C22:6n-3). RO contained 7.11% SFA and 58.71% MUFA in a total of fatty acids. There were no EPA+DHA determined in RO. The first group was fed diets with supplemented BF, the 2nd group had diets with PBE, and the 3rd group was fed with supplemented RO. The 4th group had (BF+PBE), the 5th (PBE+RO) and the 6th group were fed (BF+RO) combination of oils, all supplemented in equal amounts. Fatty acids content in diets with different fat sources is shown in Table 2. Content of fatty acids in the lipids of breast muscles was determined on 10 samples of each group by Chrompack CP-9000 chromatograph equipped with flame ionization detector (Csapo *et al.*, 1986). Portions of SFA and MUFA, as well as EPA + DHA acids were shown as a percentage of a total of fatty acids in the lipids of breast muscles. Significance of differences was determined by analysis of variance (ANOVA), using Statistica for Windows v.6.0. Differences between mean values were tested through the LSD-test ($P<0.05$).

Results and conclusions

Starting and finishing weights of turkeys, weight gains, feed consumption and conversion

Table 2. Content of fatty acids in diets with different fat sources (% of total fatty acids).

Fatty acid	Experimental groups					
	1st	2nd	3rd	4th	5th	6th
SFA	40.11	24.94	17.15	19.75	29.14	24.54
MUFA	18.19	24.15	31.25	29.86	22.36	27.02
EPA + DHA	0.00	7.39	0.00	4.07	4.09	0.00

Table 3. Fattening characteristics of turkeys (g)

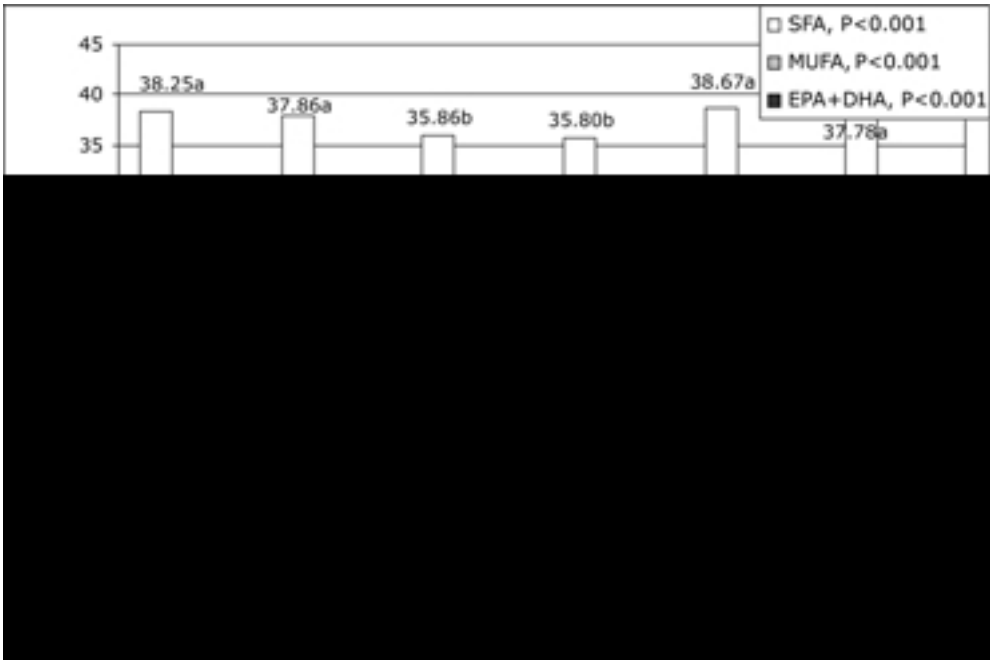
Groups	Average values per weeks of fattening				
	Starting weights	Finishing weights	Consumption	Gain	Conversion
1st	9539.87±561.72	11411.40 ± 578.16	1987± 699	468±246	4.25±0.98
2nd	9566.23±908.34	11808.69±1180.17	2193±515	561±215	3.91±0.39
3rd	9537.57±640.04	11854.17±1037.96	2207±535	582±158	3.80±0.34
4th	9461.93±573.83	11860.93± 730.83	2201±539	600±162	3.70±0.22
5th	9329.20±662.93	11655.27± 520.40	2252±370	584±109	3.86±0.16
6th	9514.07±524.28	11534.97± 686.80	2127±330	505±166	4.21±0.56
Statistical significance	P=0.757	P=0.178	P=0.831	P=0.344	P=0.275

are presented in Table 3. Different fat sources in finishing diets did not significantly affect the investigated traits in turkeys (P>0.05).

As presented in Figure 1, oils supplemented to diets and fed to turkeys had statistically very highly significant effect (P<0.001) on the content of SFA, MUFA and EPA+DHA in the lipids of breast muscles. The lowest content of SFA was found in the 4th and 3rd group (35.80% and 35.86%, respectively), being about 2% less than in other groups. As it was expected, the highest content of MUFA (23.68%) was obtained in the 3rd group of turkeys fed diets with 3% RO. Portion of MUFA being less than 20% was obtained only in the 5th group.

The highest values of EPA+DHA were determined in the lipids of breast muscles of turkeys that were given diets with added Pronova. Consequently, the content of desirable polyunsaturated fatty acids of omega n-3 type was 4.06% in the 2nd group, followed by the 4th and 5th group

Figure 1. Content of SFA, MUFA and EPA+DHA (% of total fatty acids) in the lipids of turkey breasts.



with 3.28% and 3.11% EPA+DHA, respectively. Content of EPA+DHA in turkeys fed diets with 3% BF, 3% RO and with both of those oils in combination, was 1.1%, 1.66% and 1.35%, respectively. Content of EPA+DHA in the lipids of muscles of the 2nd group was 3.69 and 2.45 times higher than in the 1st and 3rd group, respectively. In comparison with the 6th group, which received diets with 1.5% BF and 1.5% RO, supplementation of PBE combined with BF and RO in diets given to the 4th and 5th group resulted in 2.43 and 2.30 times higher portions of EPA+DHA, respectively. Rape oil in diets positively affected the content of SFA and MUFA. Diets supplemented with Pronova resulted in doubling of the EPA+DHA content, if compared to diets without it.

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SHORT COMMUNICATION

Predictability of fat-free mass and fat content in intact hen's eggs using electrical conductivity

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ABSTRACT

The effect of egg size and composition on the viability of the offspring has been tested in several former experiments, but the clarification of these correlations was mainly hindered by the lack of a reliable technique/equipment, capable of determining the egg composition *in vivo*. In the course of the present work electrical conductivity (the so-called TOBEC method) was applied for determining the fat-free mass and the fat content of intact eggs *in vivo*. Using hen's eggs in this trial it was established that for predicting the fat-free mass in hen's eggs, the weight of eggs seems to be a very good predictor (R^2=0.99) and therefore the use of the TOBEC method is not necessary in this case. In prediction of the weight and ratio of fat, the common use of the E-value (total conductivity index) and weight of eggs in the regression models resulted in a medium accuracy of prediction (R^2=0.48-0.49).

Key words: Egg, Fat, TOBEC, Electrical conductivity

Introduction

In former experiments it has already been studied whether the size or the composition of the egg had greater effect on the viability of the offspring. However, in these examinations correlations were mainly determined between different species, therefore the available information about intra-specific correlations is scarce. The clarification of these correlations was mainly hindered by the lack of a reliable technique/equipment, capable of determining the egg composition *in vivo*. In the course of the present analysis the TOBEC method (based on measuring the electrical conductivity of intact eggs) was applied for *in vivo* egg composition determination, which could be a good starting point for further examinations to clarify the correlations between egg composition and hatched birds' development.

In previous experiments the use of this method gave close correlations (r=0.88-0.99) between the E value (electrical conductivity of the whole body measured by this method) and lean mass of small birds and mammals, but only medium accuracy (r=0.59-0.66) in the prediction of total body fat percentage. Similar results were obtained using this non-destructive method for determining egg composition in different species of birds.

Based on these literature data the aim of this study was to clarify the predictability of fat-free mass and fat weight and percentage in hen's eggs to develop a useful prediction equation for the *in vivo* determination of the fat content in intact eggs.

Material and methods

The experiment was carried out with 46 hen's eggs originated from 32 weeks old HyLine Brown hens from the Experimental Farm of the University of Kaposvár. Before the measurements eggs were stored at room temperature for 24 hours for eliminating the effect of eggs' temperature on the measured values. All eggs were weighed thereafter and were positioned centrally within the detection chamber of the TOBEC analyzer in standing position. The electrical conductivity of each egg was determined once and the measured value was used for further evaluation.

The TOBEC measurements were carried out with an EM-SCAN SA-2 type Small Animal Body Composition Analyser (EM-SCAN Inc., Springfield, Illinois, USA), which allows rapid, non-invasive measurement of the total conductivity index (E value) of the eggs. This method is useful for detecting energy absorption in the presence of a radio-frequency electromagnetic field, which is created when a 10 MHz frequency is passed through a copper wire wound around a plexi-glass tube. In this system more energy is absorbed by conductive materials such as normally hydrated lean tissue than by resistant materials such as fat. With this method the fat-free mass could be measured directly and the fat content calculated from it.

After the TOBEC measurements, all eggs were analysed for fat content. Actual total lipid mass was measured using Soxhlet extraction of shell, albumen and yolk using petroleum ether. Total lean mass was then obtained by subtracting total lipid mass from total fresh egg mass.

For the *in vivo* estimation of fat-free mass and fat content of the eggs prediction equations were created by linear regression using the SPSS statistical software package (SPSS 10.0 for Windows, 1999).

Results and conclusions

As first step of the evaluation correlation between E value and fat-free mass (FFM) was examined. It was found that the E value in itself resulted in a 68% accuracy in the prediction of the weight of fat-free mass (*Equation 1*).

Equation 1: **FFM (g) = 32.4 + 0.118 x E-value (R^2=0.68, P<0.001)**

This accuracy of prediction is very similar to our former results with rabbits (Milisits *et al.*, 1999, 2000) and to the results of Williams *et al.* (1997) with eggs, but it is lower than the accuracy obtained by other authors using different species of animals.

The weight of eggs as the only independent variable in the prediction equation resulted in a significantly higher accuracy in this case (*Equation 2*).

Equation 2: **FFM (g) = -1.7 + 0.976 x Weight (g) (R^2=0.99, P<0.001)**

The inclusion of the E value into the model did not improve the accuracy of prediction (*Equation 3*).

Equation 3:
FFM (g) = -3.9 + 1.066 x Weight (g) - 0.0154 x E-value (R^2=0.99, P<0.001)

Based on these results it could be established that for predicting the fat-free mass in hen's eggs the use of the TOBEC method is not needed.

In prediction of the weight of fat the separate use of the E-value and weight of eggs resulted in a very low accuracy (*Equation 4 and 5*).

Equation 4: **Fat (g) = 1.6 + 0.0072 x E-value (R^2=0.32, P<0.001)**

Equation 5: **Fat (g) = 1.7 - 0.0245 x Weight (g) (R^2=0.08, P=0.056)**

The common use of both parameters in the model resulted in a significant increase in the accuracy of prediction in this case (*Equation 6*).

Equation 6:
Fat (g) = 3.9 – 0.0656 x Weight (g) + 0.0154 x E-value (R^2=0.48, P<0.001)

The accuracy of prediction was very similar to our former results again, where the body fat content of rabbits (Milisits *et al.*, 1999, 2000) and fishes (Hancz *et al.*, 2003) was determined.

In prediction of the fat percentage the E value and egg weight separately gave a similarly bad result as above in the case of fat weight (*Equation 7 and 8*):

***Equation 7*:** **Fat (%) = 5.0 + 0.001 x E-value (R^2=0.00, P=0.754)**

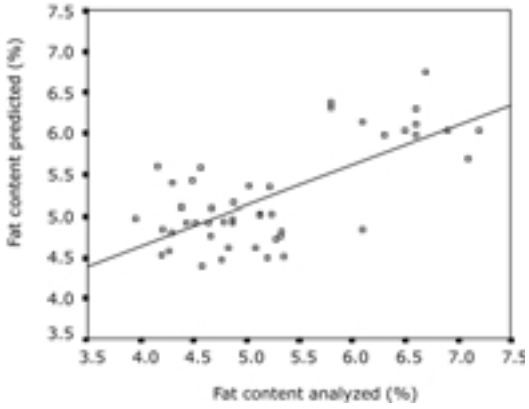
Equation 8: **Fat (%) = 8.2 - 0.0479 x Weight (g) (R^2=0.10, P=0.031)**

The common use of them, as independent variables in the equation resulted in a significant increase in the accuracy of prediction also in this case (*Equation 9, Fig. 1*).

Equation 9:
Fat (%) = 11.9 – 0.203 x Weight (g) + 0.0264 x E-value (R^2=0.49, P<0.001)

The medium accuracy of prediction obtained was also very similar to our former results on rabbits (Milisits *et al.*, 1999, 2000) and fishes (Hancz *et al.*, 2003).

Figure 1. Correlation between the analyzed and by the common use of E-value and egg weight predicted fat content.



Based on the results of this experiment it was concluded that for predicting the fat-free mass in hen's eggs, the weight of eggs, as only independent variable in the prediction equation is very reliable, therefore the use of the TOBEC method is not necessary in this case. In prediction of the weight and ratio of fat, the separate use of the E-value and weight of eggs is not reliable, but the common use of them in the regression models resulted in a medium accuracy of prediction in both cases. For improving the accuracy of these prediction equations the examination of more eggs and the use of more independent variables (for example the height and width of the eggs) seems to be necessary.

This research project was supported by a Hungarian OTKA grant (F049203).

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SHORT COMMUNICATION

Effect of Dietary Fat Level on Carcass Traits and Flesh Quality of European Sea Bass (*Dicentrarchus labrax*) from Mariculture

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ABSTRACT

The study aimed at evaluating the effect of the reduction of dietary fat on juvenile European sea bass nutritional value and quality traits. Fish were reared in floating cages (Trieste Gulf, Italy) from July (11) to October (10). Two isoproteic diets were compared: LF (low fat, EE = 19.4%) vs. HF (high fat, EE = 24.6%). No significantly different growth performance was observed. LF diet-fed fish were characterized by the reduction of celomatic fat (not edible fraction) and by the increase in dressing percentage. The tested dietary fat level also affected both fillet and epiaxial white muscle proximate composition, resulting in a significantly lower fillet lipid concentration in LF diet-fed fish. Dietary treatment influenced cooked fillet colour and texture probably as a consequence of the different intramuscular fat deposition. Fillet from HF-fed fish, in fact, presented higher lightness (L*) value and lower instrumental strengthness.

Key words: Lipids, Carbohydrates, Fish quality, European sea bass

Introduction

In order to reduce feeding cost, recent achievement on marine fish nutrition suggests the partial substitution of crude protein with lipids or carbohydrates (protein-sparing effect). However, an increase of dietary fat could result in higher fillet lipid concentration that influence organoleptic flesh quality (Lopparelli *et al.*, 2004). Indeed, the partial substitution of fish meal with starch was suggested to reduce catabolism of protein for energy with no significant effect on fish weight and whole body lipid deposition (Robaina *et al.*, 1997; Peres and Oliva-Teles 2002). When using 19% crude fat diets in European sea bass, Lanari *et al.* (1999) did not find any differences in growth and feed efficiency between 21.5% and 28.5% of dietary NFE concentration; instead, a further increase of NFE concentration led to a significantly higher whole body crude fat concentration. Considering that consumers prefer lean products, the convenience of the substitution of dietary crude protein and/or lipids with carbohydrates must consider also nutritional value and quality traits of final product. Taking this in account, it is necessary to enhance knowledge about carbohydrates utilisation by marine finfish. Thus, this trial investigated the effects of the dietary crude fat level on quality traits and proximate composition of sea bass (*Dicentrarchus labrax*) reared on floating cages.

Material and methods

Fish were reared in two floating cages (1000 m^3) near Trieste (Monfalcone, GO) under the same environmental conditions. The feeding period elapsed from July 11 to October 10, 2001. Two isoproteic (43.2% wet weight) diets were formulated varying the ether extract level: LF (Low Fat, EE = 19.4%) vs. HF (High

Fat, EE = 24.6%), and consequently increasing Nitrogen-free extract (NFE) percentage. Gross energy ranged from 21.4 to 22.5 MJ/kg. Fish were handfed until visual satiety and the administrated feed was daily recorded. At the beginning and at the end of the trial samples of fish (n=30 for each dietary thesis) were caught according to mean body weight. After dissection, the weight of body fraction was recorded. Whole body fish and skinned flesh (fillet and white dorsal muscle) were submitted to proximate composition analysis (AOAC, 2000). Raw fillet pH was determined 24 h *post mortem*. After exposition of trunks to air (1 hour at 2±1°C), raw fillet instrumental colour was determined by Hunter-L*a*b* system. After cooking during 25 min at 70°C, trunk cooking losses were recorded (Lopparelli *et al.*, 2004). In cooked fillet, colour and instrumental tenderness (Maximum Shear Force) were also assessed. According to a monofactorial design, data were submitted to ANOVA within PROC GLM of SAS (1999).

Results and conclusions

Performance data were not submitted to ANOVA because of the trial considered just two cages (one for dietary thesis). Similar feed intake and growth performances (108 vs. 103 g of weight increase) were observed throughout the feeding experimental period. Final body weight was not affected by dietary crude fat level as reported in table 1.

Dressing percentage was significantly higher for LF fish than HF ones. The result was probably due to a significant different coelomatic fat amount (19.6 vs. 22.9 g; $P<0.05$), which represents a not edible fraction. Higher dietary lipid level induced fat deposition, especially around viscera (coeloma cavity). The reduction of dietary fat was associated to a lower crude fat concentration of whole body (16.1 vs. 17.6%; $P<0.05$ – data not tabulated). On contrast, whole body humidity decreased on HF diet-fed fish, meanwhile crude protein (CP) content was similar between dietary theses. Fillet and epiaxial white muscle weights were not influenced by diets. Moreover, proximate composition of fillet showed also a higher amount of lipids on HF diet-fish (table 1), indicating that a partial substitution of dietary lipids with carbohydrates reduced intramuscular adipogenesis too. Flesh lipid data were similar to those reported from Mediterranean sea bass reared in cage and tanks (Xiccato *et al.*, 2004). No differences about fillet fatty acid chemical groups were observed (data not tabulated). Regarding flesh polyunsaturated fatty acid (PUFA), it was confirmed a higher level (35.4% of total fatty acid on average), especially for eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3). Raw fillet colour showed no differences between dietary theses, except for a* (redness). After cooking, HF-fed fish showed a fillet characterized by a higher L* (lightness) and a lower a* (table 1). An increase of the fillet fat amount is positively correlated with lightness probably as a consequence of refraction phenomenon. LF diet-fed fish showed the highest resistance and instrumental strengthness, indicating that a higher intramuscular fat deposition significantly affected maximum shear force too. Particularly, intramuscular fat deposition seems to limit collagen and muscle fibres crosslinks, reducing their mechanical strength. Decrease on texture is perceived and correlated by consumer as fatter flesh, with a reduction of acceptance and marketing value (Torrissen *et al.*, 2001). It is of interest to point out that in our trial, a decreased of dietary fat content from 24.6% to 19.4% significantly affected texture of cocked fillet but not its cooking losses.

Summarizing, a relative low substitution of energy sources from lipids to nitrogen-free extract did not affect growth performance, while significantly influenced juvenile European sea bass carcass and flesh quality traits. A decrease of dietary fat inclusion improved dressing index as a result of a lower whole body lipid deposition both as coelomatic and intramuscular fat. A different proximate composition was associated with a significant variation of fillet colour and texture properties after cooking. The use of a lower crude fat dietary concentration led to a leaner and tougher flesh, which probably better matches consumers' preference.

Financial support by MIPAF – Direzione Generale della Pesca e dell'Acquacoltura (progetto 5C 137).

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Table 1. Effect of dietary fat level (LF, Low Fat vs. HF, High Fat) on juvenile European sea bass carcass traits, proximate composition and fillet pH, colour and texture.

		July 11	October 10		P	SEM
DIETARY FAT LEVEL			LF (19.4%)	HF (24.6%)		
Weight	g	304±30	412	407	ns	6
Carcass traits						
- Dressing percentage	%	88.4±1.9	89.5	88.7	*	0.3
- Trunk weight	g	176±20	245	235	ns	4
- Viscera	"	5.7±0.7	6.1	6.0	ns	0.2
- Liver	"	5.2±1.0	11.1	10.9	ns	0.3
- Celomatic fat	"	24.4±6.0	19.6	22.9	*	1.0
Proximate composition						
Fillet						
- Moisture	%	70.6±1.1	70.0	68.8	ns	0.5
- Crude protein	"	20.1±0.4	20.7	20.6	ns	0.3
- Ether extract	"	7.7±1.4	7.7	8.9	†	0.5
White muscle						
- Moisture	"	-	73.8	72.8	ns	0.5
- Crude protein	"	-	21.5	21.6	ns	0.3
- Ether extract	"	-	3.0	4.0	*	0.3
Raw fillet						
- pH		-	6.12	6.10	ns	0.02
- Lightness	L*	42.9±1.7	45.7	46.2	ns	0.7
- Redness	a*	1.6±0.7	1.2	0.7	*	0.1
- Yellowness	b*	-2.9±0.6	-3.7	-3.4	ns	0.2
Cooked fillet						
- Lightness	L*	84.1±1.2	77.6	81.4	**	0.5
- Redness	a*	-1.6±0.3	2.5	0.5	**	0.3
- Yellowness	b*	7.1±0.4	7.6	7.3	ns	0.4
- Cooking weight losses	%	9.0±1.5	5.0	6.4	ns	0.7
- Maximum shear force	kg/cm^2	5.1±0.8	7.8	6.0	*	0.6

*†: P<0.10; *: P<0.05; **: P<0.01. Data represents means of 30 fish for carcass traits, 18 for proximate composition and 12 for reological traits.*

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SESSION III

THE ANIMAL PRODUCTION AND THE ENVIRONMENT

MAIN PAPER

Nitrogen excretion in dairy cow, beef and veal cattle, pig, and rabbit farms in Northern Italy

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Paper received April 27, 2005; accepted June 6, 2005

ABSTRACT

Reference values for N excretion of different livestock production systems are required for the application of the Nitrate Directive (91/676/EC). A survey aimed to estimate N excretion from on-farm measurements of feed consumption and performance of dairy cows (104 herds, 9,984 cows), growing cattle (40 farms, 40,157 young bulls), veal calves (34 farms, 49,206 calves), growing pigs (39 farms, 161,278 pigs) and rabbits (54 farms, 65,664 reproducing does) was conducted in Veneto from 2002 to 2003. N excretion was computed as the difference between N consumption and N retained in animal products. Dairy cow yielded 8,366 ± 1,646 kg/year of milk, consumed 6,600 ± 928 kg/year of DM, containing 2.45 ± 0.2 % DM of N, and excreted 116 ± 25 kg of N/year. No significant correlation was found between milk yield and N excretion, but the correlation between dietary N concentration and N excretion was significant (r=0.66). For growing cattle, the following mean values were achieved: daily gain 1.25 ± 0.19 kg/d; feed conversion ratio 6.9 ± 0.9 kg of DM/kg, rounds/year 1.66 ± 0.38. Nitrogen consumed, retained and excreted were, respectively, 68.7 ± 5.4, 11.4 ± 1.9 and 57.3 ± 4.9 kg/place/year. For veal calves, N consumed was 24.1 ± 1.9 kg/place/year, 12.1 ± 0.8 kg of which were retained in the body and 12.0 ± 1.5 kg were excreted. For heavy pig production, N consumed, per place and per year, averaged 19.0 ± 1.9 kg, N retained was 5.2 ± 0.5 kg and N excreted was 13.8 ± 0.4 kg. In the close-cycle rabbit farms, the doe and the relative growing rabbits (43 sold per year) consumed 11.2 ± 2.2 kg, retained 3.8 ± 0.7 kg and excreted 7.4 ± 1.5 kg N/doe/year. Nitrogen excretion estimated in this work can be considered as representative of some of the main animal production systems of the North-East of Italy. These values should not be considered as fixed, otherwise the implementation of the various strategies to reduce N excretion would not be possible. They should be considered as guidelines in the assistance both to public institutions and private enterprises in the evaluation of N excretion at farm level, favouring a more accurate quantification of the excretions, an increase of N retention efficiency and a better knowledge of the requirements of agricultural land. Moreover, a major extension of the agricultural land to be fertilised with manure should be promoted.

Key words: Nitrogen excretion, Dairy cattle, Beef cattle, Pigs, Rabbits.

RIASSUNTO

ESCREZIONE DELL'AZOTA NEGLI ALLEVAMENTI DI VACCHE DA LATTE, BOVINI DA CARNE E VITELLI A CARNE BIANCA, SUINI E CONIGLI DEL NORD ITALIA

L'applicazione della Direttiva Nitrati (91/676/EC) richiede la definizione di valori di riferimento di escrezione di N dei diversi sistemi di produzione zootecnica. Tra il 2002 e 2003 fu condotta un'indagine nella Regione Veneto per stimare l'escrezione azotata di vacche da latte (104 aziende, 9984 vacche), vitelloni (40 aziende, 40.157 capi), vitelli a carne bianca (34 aziende, 49.206 vitelli), suini (39 aziende, 161.278 capi) e conigli (54 aziende, 65.664 coniglie fattrici).

L'escrezione di N fu calcolata su base aziendale come differenza tra N consumato e N ritenuto nei prodotti animali. Le vacche evidenziarono in media una produzione di 8366 ± 1646 kg/anno di latte, un consumo di 6600 ± 928 kg/anno di s.s. di alimenti contenenti in media 2,45 ± 0,2% s.s. di N, e un'escrezione di 116 ± 25 kg N/anno. La correlazione tra produzione di latte aziendale e escrezione azotata non risultò significativa, mentre quella tra concentrazione di N della dieta ed escrezione di N fu significativa (r=0,66). Per i bovini da carne si osservarono i seguenti dati produttivi medi: accrescimento 1,25 ± 0,19 kg/d; indice di conversione 6,9 ± 0,9 kg s.s./kg, cicli/anno 1,66 ± 0,38. L'azoto consumato, ritenuto ed escreto risultò rispettivamente pari a 68,7 ± 5,4, 11,4 ± 1,9 e 57,3 ± 4,9 kg/posto/anno. Per i vitelli a carne bianca si misurarono un consumo di 24,1 ± 1,9 kg, una ritenzione corporea di 12,1 ± 0,8 kg e un'escrezione di 12,0 ± 1,5 kg N/posto/anno. Per la produzione di suino pesante, l'N consumato, ritenuto ed escreto fu in media 19,0 ± 1,9 kg, 5,2 ± 0,5 kg e 13,8 ± 0,4 kg/posto/anno. Negli allevamenti a ciclo chiuso di conigli, la fattrice con i relativi conigli prodotti (43 venduti/anno) evidenziò un consumo di 11,2 ± 2,2 kg, una ritenzione di 3,8 ± 0,7 kg e un'escrezione di 7,4 ± 1,5 kg N/anno. L'escrezione azotata stimata in questo lavoro può essere considerata rappresentativa di alcuni dei principali sistemi produttivi zootecnici del Nord-Est italiano. Questi valori non devono essere considerati fissi, altrimenti l'applicazione di possibili strategie finalizzate alla riduzione dell'escrezione azotata non sarebbe possibile. Questi dati possono tuttavia rappresentare valori di riferimento nell'attività di assistenza sia alle istituzioni pubbliche che alle imprese private nella valutazione dell'escrezione di N a livello aziendale, favorendo una più accurata quantificazione delle escrezioni, un aumento dell'efficienza di ritenzione azotata e una maggiore conoscenza dei fabbisogni di azoto dei terreni agricoli. Si dovrebbe inoltre favorire l'aumento dell'estensione dei terreni agricoli fertilizzabili con le deiezioni animali.

Parole chiave: *Escrezione azotata, Bovine da latte, Bovini da carne, Suini, Conigli.*

Introduction

An excessive application to soil of nitrogen (N) from chemical fertiliser and manure represents a threat for the quality of water, air and soil (Tamminga, 2003; Oenema, 2004). The European Directive 91/676/EC, aiming to prevent or reduce the nitrate pollution of surface and underground water, stated that each member state must identify the vulnerable areas where the load of N of livestock origin cannot exceed 170 kg/ha/year. A reliable definition of standard values for N in manure from each species and category of livestock is required for the implementation of this directive. The European Commission proposed a methodology to assist public institutions and individual producers in the computation of N in manure, taking into account the main livestock species, categories, feeding systems and management (ERM/AB-DLO, 1999). According to this methodology, N excretion is quantified as the difference between N consumption and N retention in animal products; N in manure is then calculated assuming a percentage of N lost in atmosphere during waste removal and storage. If national or regional information for the computation of N in manure is lacking, reference values proposed by ERM/AB-DLO (1999) could be used as standard values in national regulations. However, ERM reference values have been defined mainly on the basis of North Europe data and it is well known that the N in manure largely differ among European countries according to the production and feeding systems (Børsting *et al.*, 2003; Rotz, 2004). Therefore, the definition of N excretion values proper to specific local conditions is a strategic issue from environmental, economical, and social points of view.

The Italian government supported the "Interregional project on N excretion from livestock", involving Veneto, Emilia Romagna, Lombardia and Piemonte, aimed to quantify N excretion and identify the source of variation for the main national husbandry systems. This paper summarizes the main results obtained in the Veneto Region from a large survey on 271 dairy and beef cattle, pig and rabbit farms.

Material and methods

Farm data collection

Dairy cows. Data were collected in 104 dairy farms selected from the official national breeders database, representative of different breeds (60% Holstein Friesian, 20% Brown Swiss, 11% Italian Simmenthal, 9% Rendena), herd size (96 ± 63 cows/herd) and milk yield (8366 ± 1636 kg/cow/year). Dairy herds were visited by the same operator who collected feeding information according to an analytic feeding card. Data on milk yield were achieved from the official national database (AIA, 2004).

Beef cattle. Data on growth, feed consumption and diet composition were collected on 40 intensive beef cattle herds and concerned 585 production cycles involving 40,157 beef bulls of Charolaise (50%), Limousine (34%) and Polish Friesian (5%) breeds and French crossbreds (11%).

Veal calves. Data from 34 intensive veal herds involving 49,206 calves of different breeds (mainly Polish and Italian Friesian and a lesser proportion of Simmenthal and crossbreds) were used in the present study. Information was collected by the same operator who visited the farms in collaboration with public institutions and private associations.

Growing pigs. A sample of 39 heavy pig farms was identified in collaboration with various producer associations, private industries and farmers to be representative of different farm sizes, feeding techniques and territorial distribution. Detailed information concerning the farm input-output movements of feeds and animals was collected from the period 1997 to 2003. Data concerned 141 production cycles involving 161,278 pigs.

Rabbits. A sample of 54 rabbit farms was selected, representative of the Veneto distribution and management systems. Ninety percent of the farms included both reproduction and fattening sectors (close cycle), 10% of the farms had either reproduction or fattening sector (open cycle). The close-cycle units counted on average more than 1200 reproducing does with a daily presence of about 7500 growing rabbits. The average number of rabbits sold per year was 55,000, ranging from 5000 to 234,000.

Chemical analyses and nitrogen balance

Samples of rations and compound feeds were collected in each farm to be analysed for dry matter (DM) and N concentrations (AOAC, 1990). Nitrogen balance was calculated according to ERM/AB-DLO (1999). An average N concentration of milk of 0.53% was obtained from the official national database of dairy farm control (AIA, 2004). Nitrogen concentration of live weight (LW) gain was assumed equal to 2.7% for young bulls (ERM/AB-DLO, 1999), to 3.0% for veal calves (NRC, 2001), to 2.4% for growing pigs (Bittante et al., 1990; Fernández et al., 1999) and to 3.1% for growing rabbits (Szendro et al., 1998). Nitrogen in manure was calculated assuming a 28% N lost in atmosphere during waste removal and storage for all species and categories, instead of using different values (10 to 40%) proposed by ERM/AB-DLO (1999) but not proven for the waste management systems in our country as stated by the Committee of the Interregional project on N excretion from livestock.

Results and discussion

Dairy cows

Productive performance of dairy cattle, N balance and a comparison with ERM/AB-DLO (1999)

Table 1. Productive performance and N balance of dairy cattle and ERM/AB-DLO (1999) default values.

		Present study		ERM/AB-DLO
		Mean	SD	(1999)
Cows/farm	n.	96	63	-
DM consumed/cow/year	kg	6600	928	5950
N concentration of rations	% DM	2.45	0.2	2.8
Milk yield/cow/year	kg	8366	1646	7000
N concentration of milk	%	0.53	0.02	0.53
N consumed/cow/year	kg	162.1	28.2	166
N retained/cow/year	"	46.1	8.4	39.0
N excreted/cow/year	"	116.0	24.5	128
Assumed N loss	%	28		10
N in manure/cow/year	kg	83.5		114

default values are given in Table 1. Average DM consumption was 6600 kg/cow/year, considering both the milking and the dry periods. Dietary N concentration averaged 2.45% DM, and the milk yield was 8366 kg/cow/year. The amounts of N consumed, retained in animal products (milk, gain of cow and fetus), excreted and remaining in the manure averaged 162.1, 46.1, 116.0 and 83.5 kg/cow/year, respectively. When compared with the values of ERM/AB-DLO (1999) and Poulsen and Kristensen (1998), our results indicated that the average cows consumed larger amounts of DM with a lower N concentration and produced higher amounts of milk. Despite the higher DM consumption, the mean N excretion per average cow was comparable to the value proposed by ERM/AB-DLO (1999). These excretion levels can be explained considering that the diets used in Veneto dairy herds, based mainly on corn silage, presented lower N concentrations with respect to the value given by ERM/AB-DLO (1999).

The mean presence of calves and heifers in the surveyed herds approached 0.9 heads/cow. The mean annual N excretion of young animals was close to 48 kg/head, corresponding to 33.7 kg of N in manure when volatilisation was taken into account.

A large variability among herds was observed for all investigated traits. No significant correlation between milk yield and N excretion was found (r = 0.32); this probably means that the dietary N concentration is established not only considering the theoretical nutrient requirements, but also taking into account the availability of home made feed ingredients, the market prices of feeds, the need of maintaining a low level of urea in blood and in milk. Conversely, a significant correlation (r = 0.66) was found between dietary N concentration and N excretion/cow/year, confirming that the reduction of the dietary protein is a main strategy for reducing N excretion from dairy farms (Børsting *et al.*, 2003).

Beef cattle

The initial and final LW averaged 340 and 600 kg, the daily weight gain was 1.25 kg/d, feed conversion ratio was slightly lower than 7.0 and the mean N concentration of rations was 2.31% DM (Table 2). Nitrogen consumption averaged 68.7 kg/place/year, N retention 11.4 kg/place/year and N excretion 57.3 kg/place/year. The value of N excretion was similar to that found by Smith and Frost

Table 2. Productive performance and N balance of beef cattle and ERM/AB-DLO (1999) default values.

		Present study		ERM/AB-DLO (1999)
		Mean	SD	
Place/farm	n.	412	399	
Initial LW	kg	342	62	
Final LW	”	608	78	
Average daily gain	kg/d	1.25	0.19	1.0
Rounds/year	n.	1.66	0.38	
DM feed conversion ratio	kg/kg	6.91	0.87	7.4
N concentration of rations	% DM	2.31	0.15	2.7
N concentration of LW gain	%	2.7		2.7
N consumed/place/year	kg	68.7	5.4	70
N retained/place/year	“	11.4	1.9	7
N excreted/place/year	“	57.3	4.9	63
Assumed N loss	%	28		10
N in manure/place/year	kg	41.3		

(2000) for large-size growing cattle of 1 to 2 years of age. The standard deviation of N excretion, close to 9%, was comparable with that reported by Poulsen and Kristensen (1998) and Smith and Frost (2000). A more detailed analysis, not reported here, evidenced significant differences due to the breed (Gallo *et al.*, 2004). Nitrogen excretion found in this research was about 10% lower than the value of 63 kg/place/year proposed by the ERM/AB-DLO (1999). Also in this case, this result can be ascribed to the lower N concentration of the rations with respect to the value of 2.8% DM indicated by ERM/AB-DLO (1999). This low dietary protein concentration can be explained by the widespread application of the total mixed ration technique, the frequent changes of dietary composition according to age (the number of rations in the growing period was 3.6), as well as the high proportion in the rations of corn silage, sugar beet pulp, cereals and the limited amount of soybean meal and legumes hay.

Veal calves

Places per herd averaged 681 with 2.1 cycles/year. As reported in Table 3, initial and final LW averaged 61 and 253 kg, respectively, and average daily gain was 1.19 kg/d. Performances were comparable with those previously reported by others (Andrighetto *et al.*, 1996; Xiccato *et al.*, 2002). The mean N concentration of the diet, mainly based on milk replacers, was 3.36% DM. Nitrogen concentration of LW gain was assumed to be 3.0% on the basis of previous comparative slaughter experiments (Andreoli *et al.*, 1996). Nitrogen consumption amounted 24.1 kg/place/year and 12.1 kg were estimated to be retained in the body; therefore, average N excretion was 12 kg/place/year. Assuming a N loss of 28%, the resulting N in manure was 8.6 kg/place/year. No data of N balance is provided by ERM/AB-DLO (1999) or other literature for veal calves. The resulting N retention efficiency (50%) is explained by the low feed conversion ratio (1.73 kg DM/kg) and it is comparable with values found for other monogastric species at young ages.

Growing pigs

Pig sector in Italy is peculiar with respect to other European countries, since it is driven by the production of certified (DOP) cured ham. The heavy LW (160 kg or more) and the minimum age (9 months) at slaughter imposed by DOP regulation

Table 3. Productive performance and N balance of veal calves (no default values are given by ERM/AB-DLO, 1999).

		Present study		ERM/AB-DLO
		Mean	SD	(1999)
Place/farm	n.	681	540	
Initial LW	kg	61	6.1	
Final LW	"	253	13.9	
Average daily gain	kg/d	1.19	0.06	
Cycles/year	n.	2.1	0.1	
DM feed conversion ratio	kg/kg	1.73	0.10	
N concentration of ration	% DM	3.36	0.03	
N concentration of LW gain	%	3.0		
N consumed/place/year	kg	24.1	1.9	
N retained/place/year	"	12.1	0.8	
N excreted/place/year	"	12.0	1.5	
Assumed N loss	%	28		
N in manure/place/year	kg	8.6		

strongly affect feeding practices and diet characteristics. Although this production has a large economic relevance, few systematic data on production traits and N balance are available (Schiavon *et al.*, 1997).

The main production traits and N balance are given in Table 4 and compared with values proposed by ERM/AB-DLO (1999) for other pig production systems (light pigs). Even though relevant differences for all production variables were observed, N excreted per place and year was similar to the values proposed by other references (ERM/AB-DLO, 1999; Fernández *et al.*, 1999; Smith *et al.*, 2000) and close to 13.5 kg. Restricted feeding and low protein diets used in heavy pig production are aimed to maintain average LW gain around 0.640 kg/d during the whole growth period and to avoid the risk of pigs too young and lean at the target slaughter weight (Bosi and Russo, 2004). Some studies showed the feasibility of a further reduction of N excretion by decreasing the dietary protein level and optimizing the amino acid profile (Piva and Mordenti, 1995). Performance and N balance concerning sow herds with or without piglets in the Veneto conditions (Ceolin *et al.*, 2005; Tagliapietra *et al.*, 2005) were in agreement with ERM/AB-DLO (1999).

Rabbits

The main productive data and N balance are given in Table 5. In the close-cycle farms, the N balance is referred to the reproducing doe (4 kg LW) and its offspring (43 rabbits/year sold at 2.5-2.6 kg). The doe and the relative growing rabbits consumed 11.2 kg N/doe/year and retained 3.8 kg N/doe/year. The doe and its offspring excreted yearly 7.4 kg N, that is 20% lower than the value proposed by ERM/AB-DLO (1999) and very close to the results of Maertens *et al.* (2005). ERM/AB-DLO (1999) assumed a 40% N loss in atmosphere, against 28% of our study, therefore suggesting similar quantity of N in manure (5.5 kg N/doe/year for ERM/AB-DLO and 5.3 kg for our study). When N excretion was expressed on different productive units, excreted N was 0.172 kg/sold rabbit and 0.069 kg/kg sold rabbit. These values are somewhat lower than those proposed by ERM/AB-DLO (1999), i.e. 0.184 kg/sold rabbit or 0.088 kg/kg sold rabbit, but differences can be at least partly explained considering different assumptions for N loss during manure removal and storage.

In the reproduction open-cycle farms with rabbit does weaning 47 kits per year at 0.9 kg LW, excreted N was 2.5 kg/doe/year, that is 1.8 kg/doe/year in manure. In the fattening open-cycle farms, with weaned rabbits bought at 0.9 kg LW and sold at 2.5 kg and 7 fattening cycles/year, excreted N resulted 0.80 kg/fattening place/year, that is 0.58 kg/fattening place/year in manure. Nitrogen excretion referred to the reproducing does was influenced mainly by the rabbit LW at slaughter (2.4 to 2.9 kg in the surveyed farms) and by the number of sold rabbits (35 to 60 per doe/year). Differently, total N excreted by the farm was main-

Table 4. Productive performance and N balance of growing pigs and ERM/AB-DLO (1999) default values.

		Present study		ERM/AB-DLO
		Mean	SD	(1999)
Place/farm	n.	1289	1037	
Initial LW	kg	28.5	4.7	25
Final LW	"	163.4	5.3	105
Cycles/year	n.	1.60	0.17	3.0
Feed conversion ratio	kg/kg	3.64	0.26	2.9
N concentration of feeds	% DM	2.73	0.11	2.8
N concentration of LW gain	%	2.4	2.5	
N consumed/place/year	kg/place/year	19.0	1.87	19.5
N retained/place/year	"	5.19	0.46	6.0
N excreted/place/year	"	13.81	0.44	13.5
Assumed N loss	%	28		26
N in manure/place/year	"	9.96		10.1

Table 5. Productive performance and N balance of rabbits (reproduction + fattening farms) and ERM/AB-DLO (1999) default values.

		Present study		ERM/AB-DLO
		Mean	SD	(1999)
Reproducing rabbits/farm	n.	1216	885	
Rabbits sold/year/farm	"	54,441	44,357	
Final LW	kg	2.65	0.11	2.10
Feed conversion ratio	kg/kg	3.82	0.19	4.00
Rabbits sold/doe/year	n.	42.8	8.7	50.0
LW rabbit sold/doe/year	kg	113.1	22.4	105.0
N concentration of feeds	% DM	2.88	0.11	2.94
N concentration of LW gain	%	3.1		3.0
Feed intake/doe/year	kg	431	81	420
N consumed/doe/year	"	11.17	2.16	12.3
N retained/doe/year	"	3.77	0.70	3.1
N excreted/doe/year	"	7.40	1.48	9.2
Assumed N loss	%	28		40
N in manure/doe/year	kg	5.33		5.5

ly influenced by the number of reproducing does, even if the estimation of farm N excretion was improved when the total weight of rabbits sold per year was included in the predicting equation. Nitrogen excretion values calculated in this study were notably higher than the values currently assumed by Italian national and regional legislation. An overall decrease of dietary N concentration and the application of feeding plans characterized by protein decreasing with age may permit a substantial reduction of N excretion without impairment of growth performance and meat quality (Maertens *et al.*, 1997; Trocino *et al.*, 2000).

Conclusions

Several strategies aimed to reduce the excretion of N and other nutrients from livestock have been proposed in the literature for different species (Piva and Mordenti, 1995; Maertens *et al.*, 1997; Satter *et al.*, 2002). All these strategies aim to increase the efficiency of retention of the dietary nutrients in animal products. The reader can found more details in the reviews of Tamminga (1996) and Maertens (1999).

The promotion of these strategies at practical level is strictly depending on the constraints settled by regulations. In our opinion, the adoption of reference coefficients for computing farm N excretion based exclusively on the number of heads would not promote the on-farm application of strategies aimed to reduce the excretion per head. This kind of approach simply acts to reduce the number of livestock unit/ha, with serious risks of a strong decrease in the number of livestock farms, particularly in the areas vulnerable to nitrate pollution.

The results of our study can contribute to a more precise estimation of nitrogen excretion of livestock as a function of productive management and feeding systems, and in particular:

1) the values of N excretion determined in the present study are representative of some livestock production systems currently applied in the Northern Italy and can be useful as reference val-

ues to setup regional or national regulations;

2) for some species, namely dairy cows, beef cattle and rabbits, the average values of N excreted are somewhat higher than the values currently assumed by local regulations. Therefore, in many areas there could be an excess of N from manure, while in other cases the exclusive use of chemical fertilizer results in a reduction of the organic matter content of the soil, with negative consequences on its structure, texture and fertility;

3) the adoption of farm protocols for recording feed consumption, feed composition and production performance would promote a better identification of the critical points of husbandry and a more accurate quantification of excretions as well as the need of agricultural land;

4) the methodological approach followed in this study and the set of values found for the different traits of production and N balance, both in terms of mean and variability, can be used as reference for the assistance in the evaluation and control of N excretion at farm level.

In conclusion, besides the increase of agricultural land involved in the use of manure, the application of feeding and management techniques capable of increasing N retention efficiency and reducing N excretion per animal product unit should be promoted. Moreover, a better definition of the chemical and physical characteristics of manure, as a result of feeding and housing systems and manure removal, treatment, storage, is also needed in order to promote the correct application of animal waste on agricultural land and to reduce the risks of environmental pollution.

The Authors wish to thank Dr. Valerio Bondesan (Veneto Agricoltura, Legnaro, Padova) for his technical assistance in all aspects of the research.

Research supported by Veneto Region and coordinated by Veneto Agricoltura (Interregional research programs, Law 499 of 23 Dec. 1999).

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SHORT COMMUNICATION

Simulation of growth performance of heavy pigs with different inherent growth characteristics kept on two restricted feeding planes

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ABSTRACT

A mechanistic mathematical model was run to simulate the effect on average daily gain (ADG) and feed conversion ratio (FCR) of two different kind of pigs and two different feeding regimes. Theoretical values, taken from literature, were used as inputs to describe a poor and a improved genotype. To describe the feed, experimental measurements of feed composition and feed consumption, taken over 5 consecutive periods of growth from two groups of pigs, from 25 to 160 kg of live weight, were also used as inputs. The simulated ADG and FCR values were compared with the experimental performance. Across periods the experimental pigs showed ADG and FCR within the simulated ranges obtained for the theoretical pig genotype. It was concluded that the model was sensible to the variations of pig genotype and of feeding regime. It provided reasonable response both in term of pattern and magnitude.

Key words: Heavy pigs, Mathematical model, Growth performance, Potential growth

Introduction

A number of mathematical models have been developed with the purpose of predicting the growth and the feed intake of pigs kept under different feeding and environmental conditions (Moughan *et al.*, 1987; Ferguson *et al.*, 1994). The model proposed by Ferguson *et al.* (1994) was considered by ASPA (2003) as one of the more interesting for possible application in the Italian heavy pig production system. This model predicts the chemical growth in non limiting and limiting conditions, as result of the interaction of the following 3 components: the pig potential chemical growth, the feed availability and feed composition and the environment. One of the major problem for the application of the model in the practice is the quantitative evaluation of the first component (Emmans, 1989) which can be described by only 3 variables: the protein weight at maturity (P_m), the maturing rate (B) and the lipid to protein ratio at maturity (L_m/P_m). Feed is described in terms of day by day feed allowance and by the feed concentration of the effective energy and ideal protein (Emmans, 1994). The environmental temperature is used to describe the third component (Schiavon and Emmans, 2000). On the basis of the theoretical concepts of this model it is expected that pigs with different inherent potential growth respond differently when kept on the same feeding regimes. Thus, the aim of this paper was to evaluate the pattern and the magnitude of response of the model, in term of average daily gain (ADG) and feed conversion ratio (FCR), of 2 groups of pigs with different potential growth receiving 2 different feeding planes. The responses of the model were tested against experimental data.

Material and methods

The model was run using inputs to describe the pig, the feed and the environment. To describe the pig, 2 sets of theoretical values, suggested by Whittemore *et al.*

Table 1. Amount of feed consumed (kg/period) by the pigs receiving the 2 different feeding planes.

Feeding plane	0-41 days	41-62 days	62-97 days	97-125 days	125-194 days	0-194 days
Low-High	56.7	39.9	80.2	71.6	197.1	445.5
High-Low	64.4	47.3	77.0	66.6	192.6	447.9

(2003) were used: for a poor pig genotype the value of P_m, B and Lm/Pm were assumed 30 kg, 0.011 d^{-1} and 4, respectively; for an improved genotype the values of P_m, B and Lm/Pm were assumed 40 kg, 0.0115 d-1 and 4, respectively. To describe the feed experimental measurements, about feed consumption and feed composition, of two groups of 15 commercial crossbreed pigs receiving two different feeding regimes, were used. In this experiment the two groups of pigs were housed in two pens of the same room from 25 to 160 kg of live weight (LW) and received different amount of the same feeds in 5 consecutive periods (treatment: Low-High and High-Low). Feed consumption and LW were measured at 41, 62, 97, 125 and 194 days from the beginning of the trial. Group LH received a larger amount of feed in the first part of the trial, the reverse was for the HL group (Table 1). The chemical composition of the 4 feeds used is given in Table 2. To describe the environment a thermo neutral temperature of 20°C was used. The model was run 4 times (2 genotypes x 2 feeding planes) simulating day by day daily gain (ADG) and feed conversion ratio (FCR) and averaging these values for each of the 5 periods of controls. The simulated response were compared with the corresponding experimental measurements.

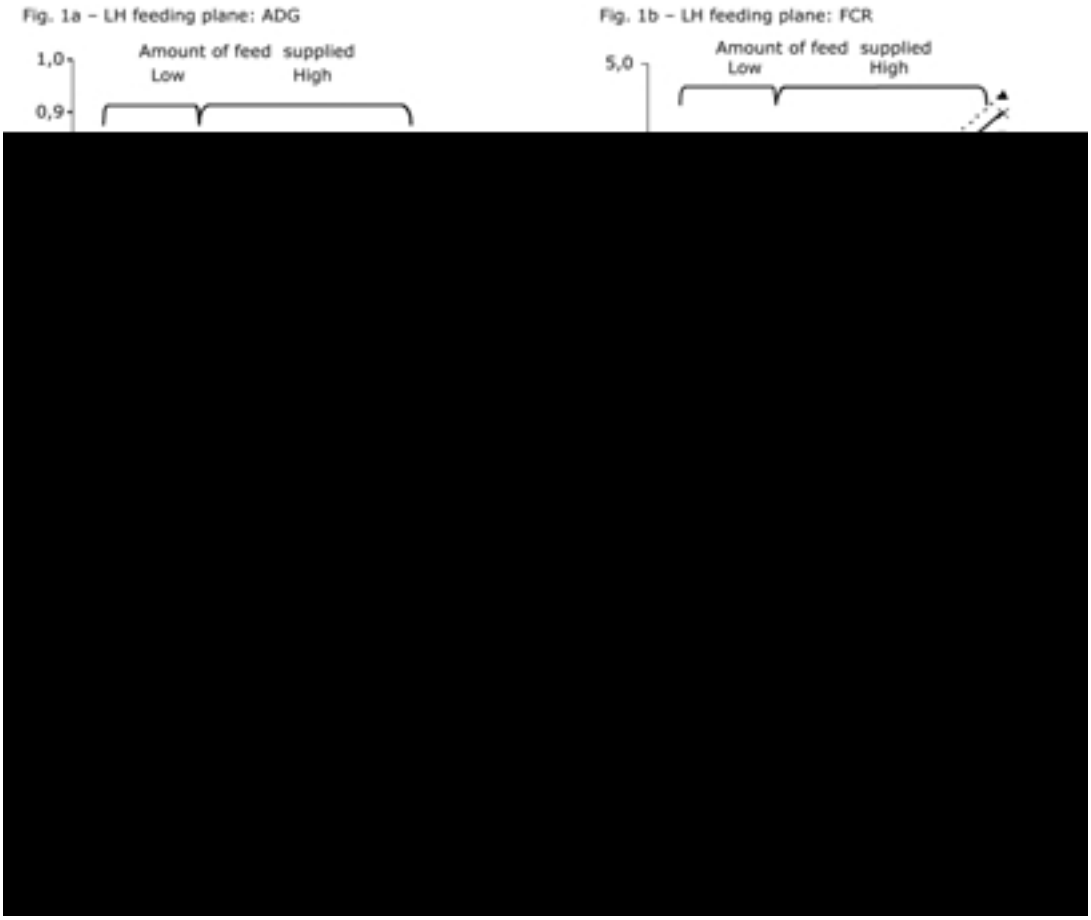
Results and conclusions

The simulated and the measured values of ADG and FCR for the LH feeding plane are given in the figures 1a and 1b. The pattern and the magnitude of response of the model agreed well with the experimental data. The average values of ADG and FCR of the experimental pigs (0.69 kg/d and 3.31 kg/kg) were within the range defined by the poor (0.66 kg/d, 3.45 kg/kg) and the improved genotypes (0.72 kg/d, 3.19 kg/kg). Higher ADGs corresponded to lower FCRs, reflecting differences in the chemical composition (protein, water, ash and lipid) of LW gains. In the second part of the growth, when feed availability was higher, the experimental pigs grew faster than the simulated poor pig genotype, but slower than the improved one. The values of ADG and FCR obtained for the HL feeding plane are shown in the figures 2a and 2b. Considering the form of the curves, the model outputs were sensible to the change of feeding regime. With respect to experimental pigs, the improved pig genotype presents an higher ADG during the first part of the growth, when the feed availability was higher (0.79 kg/d *vs.* 0.72 kg/d). The corresponding values of the FCR (2.3 kg/kg *vs.* 2.5 kg/kg), reflect a different ability to retain protein, and consequently, water and ash, in the body. The early growth of the experimental pigs was similar to that achieved for the poor pig genotype (0,72 kg/d *vs.* 0,70 kg/d), and did not appear to be marked affected by the higher feed availability. However, the later growth of experimental pigs was higher than that simulated for the poor genotype (0.68 kg/d *vs.* 0.65 kg/d).

Table 2. Amount of feed consumed (kg/period) by the pigs receiving the 2 different feeding planes.

Periods		1-28 days	29-77 days	78-126 days	127-194 days
		Feed 1	Feed 2	Feed 3	Feed 4
DE	MJ/kg dm	15.43	15.18	15.05	14.65
EE	"	13.63	13.36	13.25	12.79
DCP	% dm	16.68	15.02	14.28	13.28
Lys	"	1.01	0.78	0.74	0.66
Met+Cys	"	0.58	0.46	0.43	0.39
Thr	"	0.60	0.52	0.44	0.39

DE: digestible energy; DCP: digestible crude protein; EE: effective energy; Lys: digestible lysine; Met+Cys: digestible methionine + cysteine, Thr: digestible threonine.



It can be concluded that the patterns and the magnitude of the model response were reasonable and acceptable. The model was sensible to changes of pig genotype and feeding regime, it provided reasonable results, comparable with the experimental ones. The major shortcomings in the application of this model remains the availability of operational values to describe the pig genotype. However, the results obtained in this work suggest that the potential growth characteristics of the experimental pigs were probably intermediate to the theoretical values assumed for the two simulated genotypes.

The authors are grateful to Veronesi S.P.A. for the providing the experimental data.

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SHORT COMMUNICATION

On-farm estimation of pig growth parameters from longitudinal data of live weight and feed consumption and the use of a mathematical model

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ABSTRACT

Experimental data of feed consumption and composition, recorded over 5 consecutive short periods of growth from 30 crossbred male pigs divided 2 two groups and fed two restricted feeding regimes from 25 to 160 kg LW, were used to run a model in which a set of theoretical values, to describe the potential chemical growth of the pig, was preliminarily assumed. Simulated values of average daily gain (ADG) and feed conversion ratio (FCR) were the outputs. Estimates of protein mass at maturity (Pm, kg) and relative growth rate (B, d^{-1}) for each feeding regimes where successively obtained through an optimization procedure which has minimized the coefficients of variation of the differences between the estimates and the measurements of ADG and FCR. The very similar values of Pm (33.7 and 33.2 kg), and B (0.0104 and 0.0105 d^{-1}), obtained for the two feed treatments suggested that these values can be used as operational data to describe the growth parameters of the experimental pigs used.

Key words: Mathematical model, Growing pigs, Inherent growth characteristics

Introduction

The major constraint in the application of mathematical model in the practice is the description of the pig (Emmans, 1989; Schiavon *et al*., 2004). In the Ferguson *et al.* (1994) model the potential growth of the pig is described in term of mature protein mass (Pm, kg), rate of maturing (B, d^{-1}) and lipid to protein ratio at maturity. Ceolin *et al.* (2005) run this model using a) 2 sets of theoretical values to describe a poor and a improved pig; b) longitudinal data about feed consumption and composition, measured over 5 consecutive short periods of growth, of 30 commercial crossbred pigs divided in 2 groups fed two different restricted feeding regimes. The simulated model responses in term of average daily gain (ADG) and feed conversion ratios (FCR), for each of the 5 growing periods, were compared with the measured ones with good results. The values of Pm and B of these pigs were probably intermediate to the two theoretical sets of values assumed. Thus, in this paper an optimization procedure was proposed in order to evaluate the operational values for the growth parameters of Pm and B of this group of pigs.

Material and methods

The model of Ferguson *et al.* (1994) was run using as input to describe the feed experimental data of a group of 30 commercial crossbreed pigs, which were divided in two groups (HL and LH) and fed, from 25 to 160 kg of LW, different amounts of feeds in 5 consecutive periods. With respect to the HL group, the LH group received in the early and in the later period of growth a lower and a higher amount of feeds, respectively. In table 1 the amounts of feeds consumed in different periods are shown. The 2 groups of pigs received, but in different amounts, the same 4 feeds, which contained 13.6, 13.4, 13.3, and 12.8 MJ/kg of effective energy (EE), 16.7, 15.0, 14.3 and 13.3% of digestible crude protein, and 1.01, 0.78, 0.74 and 0.66% of digestible lysine (mayor detail are given by Ceolin et. al., 2005). A set of theoretical values for Pm, 30 kg, B,

Table 1. Growth periods (days), amounts of feed consumed (kg/period) and actual and simulated values of final live weight (FLW, kg), average daily gain (ADG, kg/d) and feed conversion ratios (FCR) for the 2 feeding treatment using the optimized values of Pm and B.

	Days	Actual feed consumed	FLW		ADG		FCR	
			Actual	Simulated	Actual	Simulated	Actual	Simulated
Group LH	0-41	56.7	51.0	50.7	0.617	0.610	2.240	2.267
	41-62	96.6	67.0	66.2	0.762	0.736	2.498	2.573
	62-97	176.8	94.5	93.5	0.786	0.781	2.915	2.934
	97-125	248.4	115.7	115.0	0.757	0.766	3.377	3.329
	125-194	445.5	160.3	160.0	0.646	0.653	4.420	4.358
Final CV%			0.8		2.2		1.8	
Group HL	0-41	64.4	53.0	52.1	0.688	0.665	2.286	2.37
	41-62	111.7	69.6	69.4	0.790	0.826	2.847	2.71
	62-97	188.7	94.2	95.6	0.703	0.749	3.131	2.94
	97-125	255.3	113.2	115.1	0.678	0.695	3.507	3.41
	125-194	447.9	159.5	159.0	0.672	0.636	4.156	4.38
Final CV%			1.3		5.2		5.5	

Initial live weight of groups LH and HL were 25.7 and 24.8 kg, respectively; CV%= coefficient of variation of the differences between the actual and simulated values after optimization.

0.0104 d^{-1}, and Lm/Pm, 4, was preliminary used to describe the pig and thermo-neutral conditions were assumed. On the first run the model computed, day by day, for each feed treatment, the values of ADG and FCR. These daily values were averaged to be representative of each period of growth and then compared with the measured ones. Within each feed treatment, the coefficients of variation (CV) of the differences between simulated and measured values were computed. An optimization procedure was applied to find the values of Pm and B which make minimum the values of CV of ADG. The model was run a second time with the new Pm and B values and the corresponding new estimates of ADG and FCR were compared, by linear regression, with the corresponding actual measurements. The good correspondence between actual and estimated values of ADG and FCR allowed to proceed to an evaluation of the growth of each chemical constituent over the whole experimental period.

Results and conclusions

The values of Pm and B, obtained after the optimization procedure, were quite similar: 33.7 and 33.2 kg and 0.0104 and 0.0105 d^{-1} for the LH and the HL groups, respectively. The values of Pm and B were in the range observed by others (Emmans and Kyriazakis, 1999; Whittemore *et al.*, 2003). The two groups of pigs showed, in each period, different final live weights (FLW), ADG, FCR according to the different feeding planes adopted (Table 1).

The linear regression between actual (a) and simulated (s) values of FLW, ADG and FCR were:

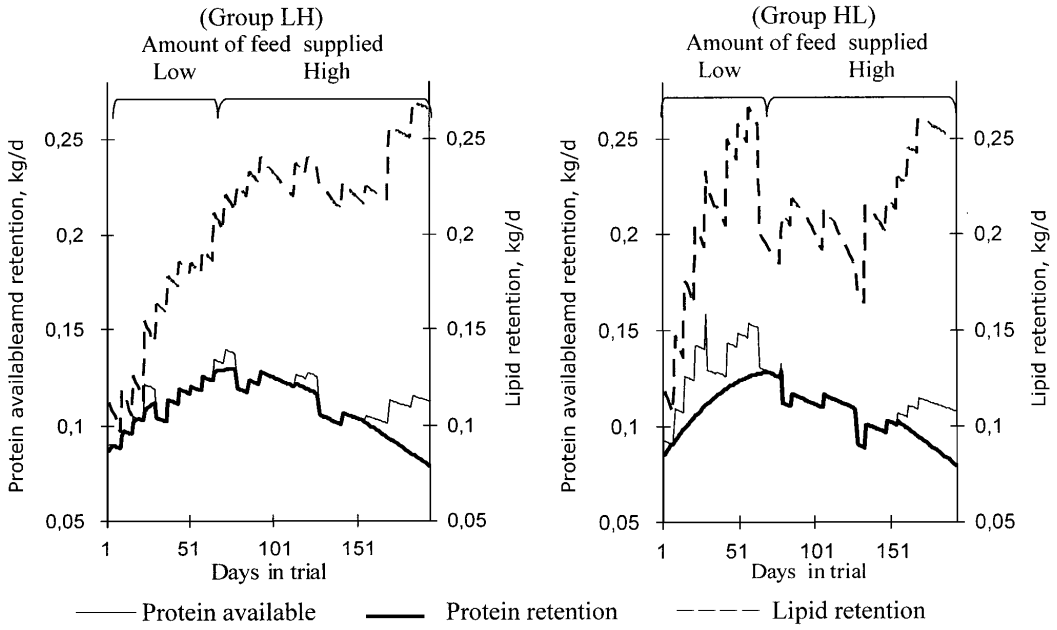
a) $FLW_a=1.001*FLW_s$ (10 obs., $R^2= 0.9994$, rsd = 1.0);

b) $ADG_a=0.996*ADG_s$ (10 obs., $R^2=0.8629$; rsd= 0.026);

c) $FCR_a=1.002*FCR_s$ (10 obs., $R^2 = 0.9737$; rsd= 0.122).

For all these regressions the values of the parameters reflected a good correspondence between the model outputs and the measurements. The simulated evolution over the time of the chemical composition of gain, for the two feeding plane, is shown in Figure 1. The continue and bold lines represent the predicted protein retentions, while the dotted lines represent the lipid retention. It can be observed that, according to the availability of net protein for growth (continue line), the model predicted some deviations as respect to the potential protein growth, in particularly for the HL feeding treatment. Excess of protein supplies were estimated for the early period for the HL treatment and for the last period for both the two treat-

Figure 1. Evaluation of the consumption of net protein available for growth and of the expected daily retention of protein and lipids. The graphics show the predicted curves for the group LH and HL respectively.



ments. In conclusion, the approach followed in this work allowed to obtain some estimates of the parameters required to describe "the pig growth characteristics" under on-farm conditions which can be considered as operational values for this kind of pigs. Even though more test are required, the results obtained in this work suggest that the approach based on simple measurements of feed consumption and live weight, can be applied to a mathematical model to approximate the inherent growth characteristics of a group of pig. Many benefits can be achieved from the possibility of using a model in the farm: a better definition of the feeding standards for a given kind of production, the possibility of predicting changes of body composition when different feeding regime are applied, the possibility of studying the effects of different genotype on the evolution of body composition under different feeding regimes. It is desirable that the mathematical model will developed in the future in order to establish a link between the farmer and the industry for optimizing the feeding practices, to improve the quality of the products and to reduce the excretion of nutrients in the environment.

The authors are grateful to Veronesi S.P.A. for the technical support.

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SHORT COMMUNICATION

Blood serum metabolites and meat quality in crossbred pigs experiencing different lairage time

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ABSTRACT

Pigs of two crosses: A (♀Duroc x ♂Swedish Landrace) x ♂Pietrain (n=24) and B (♀Swedish Landrace x ♂Large White) x ♂Pietrain (n=26) were used to investigate the effects of different lairage time (2 and 24 hours) on levels of stress and meat quality traits. No direct effect of lairage time on cortisol, lactate, electrolytes and meat quality parameters was observed. However, after long lairage time, pigs showed lower level of glucose and higher CK, AST and ALT activity. Crossbred B pigs exposed to short lairage time, showed higher blood lactate, sodium, and potassium level, higher drip loss and lower pH_i, whereas there were no significant differences between the crossbreeds in the long lairage group. The results indicate that long lairage time decreases blood glucose level and produces signs of muscle damage. In the short lairage period, the crossbreed B showed a higher response to pre-slaughter handling affecting the meat quality.

Key words: Lairage time, Crossbreed, Blood metabolites, Meat quality

Introduction

The pre-slaughter handling of pigs include removal from the familiar fattening pen, loading, transportation, unloading, mixing with unfamiliar pigs, overcrowding and exposure to novel environmental conditions. The behavioral and physiological changes associated with handling, have been found in earlier investigations (Warris *et al.*, 1998; Fabrega *et al.*, 2002) indicating that all these procedures may impose stress which impairs the welfare and meat quality. Physiological responses to pre-slaughter stress in pigs is associated with changes in plasma cortisol, glucose, lactate, white blood cell (WBC), packed cell volume (PCV), electrolytes, plasma osmolality as well as creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity (Schaefer *et al.*, 1997; Perez *et al.*, 2002). Pre-slaughter stressors also change muscle glycogen metabolism and affect meat color, water holding capacity (WHC) and technological traits (Gispert *et al.*, 2000). Short pre-slaughter stress may cause a rapid fall in muscle pH *post mortem* and the development of pale, soft and exudative (PSE) meat. Long term stress conditions cause muscle fatigue and depletion of muscle glycogen at slaughter, producing dark, firm and dry (DFD) meat. The incidence of PSE and DFD meat is largely influenced by the genotype of the pig (Gispert *et al.*, 2000) and lairage time (Santos *et al.*, 1997). Precise period of rest and its influence on levels of stress is not well defined. Using the changes in the blood profile as an indicator, Warris *et al.* (1998) found that the overnight lairage reduced the amount of stress. Perez *et al.* (2002) observed that the absence of

Results and conclusions

Least square means (standard errors) of the blood and meat quality parameters from the two crossbreeds within short and long lairage time are given in Table 1.

The results in Table 1. showed that cortisol levels were high in all the groups in comparison to preload levels (mean preload levels of cortisol were 2.52 and 2.61 in the short lairage group and 2.34 and 2.17 in the long lairage group, for A and B crossbreed, respectively), indicating stress response activation. This finding is in the agreement with earlier investigations (Kannan

Table 1. Effects of lairage time and crossbreed on blood and meat quality parameters.

Lairage time		Short - 2 h		Long - 24 h	
Crossbreed		A	B	A	B
Blood parameters					
Cortisol	mmol/l	8.43(0.56)	8.28 (0.59)	7.66 (0.54)	8.59 (0.54)
Lactate	"	10.54 (0.80)[a]	14.74 (0.84)[b]	8.44 (0.76)[a]	8.47 (0.76)[a]
Glucose	"	6.70 (0.19)[a]	7.25 (0.20)[a]	5.16 (0.18)[b]	5.35 (0.18)[b]
CK	(U/L)	9206 (3073)[a]	10939 (2898)[a]	26763 (2749)[b]	22787 (2622)[b]
LDH	"	3420 (234)	3084 (234)	2679 (222)	1942 (211)
AST	"	76.08 (7.35)[a]	72.83 (7.75)[a]	139.06 (7.35)[b]	118.54 (7.01)[b]
ALT	"	53.96 (3.24)[a]	57.49 (3.29)[a]	72.75 (3.35)[b]	75.27 (3.25)[b]
Potassium	mmol/l	6.58 (0.28)[a]	8.24 (0.30)[b]	6.65 (0.27)a	6.51 (0.37)[a]
Chloride	"	99.96 (0.57)	101.14 (0.60)	100.96 (0.54)	101.38 (0.54)
Sodium	"	145.96 (0.98)[a]	151.74 (1.0)[b]	148.48 (0.93)[a]	148.37 (0.93)[a]
Meat quality					
pH_i		6.47 (0.08)[a]	6.07 (0.07)[b]	6.09 (0.08)[b]	6.10 (0.08)[b]
pH_u		5.63 (0.04)	5.56 (0.03)	5.66 (0.04)	5.61 (0.04)
Drip loss ()	%	5.56 (0.61)[a]	7.79 (0.59)[b]	6.32 (0.66)[a]	6.24 (0.66)[a]

value. In the short lairage group, the crossbreed B showed a lower pH_i value ($P<0.01$) and a higher drip loss ($P<0.05$) compared with crossbreed A, whereas crossbreed did not alter the pH_i or the drip loss in the long lairage group. Elevated blood lactate levels originate from the breakdown of the muscle glycogen as a result of extreme muscular activity and from the catecholamine release leading to rapid glycogenesis. Accordingly, higher blood lactate level in the crossbreed B may indicate a higher response to physical exercise and psychological stress in the immediate pre-slaughter period. Such thesis is supported by the tendency of developing rapid muscle acidification and consequently a higher drip loss observed in the same group. The results of the present experiment indicate that long lairage time decreases blood glucose level and produces signs of muscle damage. A different reaction to pre-slaughter handling between crossbreeds was observed only in the short lairage period with a higher response to pre-slaughter stress and poor meat quality in the crossbreed B.

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SHORT COMMUNICATION

Analysis of factors affecting health status of animals under intensive beef production systems

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ABSTRACT

From 2001 to 2003 health data of 588 groups of fattening beefs (38723 animals) of 29 farms and 5 genetic types (Charolais, Female Charolais, French Crosses, Limousine and Polish Friesian) were collected and statistically analysed considering farm, breed, initial body weight, housing system, season, year, diet, average daily gain and density. The main causes of culling were problems of the locomotor and respiratory systems. As regards the genetic type and the initial body weight, the Polish Friesian breed and the lightest weights increased the probability of dying or getting injured respectively of 70 and 34%, while this probability decreased of 8.5% for every 100 g of daily growth higher than the mean value. As regards the other factors, bedding reduced the risk of culling by 33% in comparison with the slatted floor, while this risk increased of 10% for every m^2 available/animal over the mean density.

Key words: Health problems, Intensive production system, Beef

Introduction

The present Italian annual bovine meat consumption is about 1.43 million tons, i.e. about 24 kg/year/citizen. Only 60% of the beef consumption comes from animals slaughtered in the country (ISTAT, 2002) and the main regions where bovine meat production takes place are Piemonte, Lombardia, Veneto and Emilia Romagna. The Veneto region (North-eastern Italy) contributes to a 13% of the beefs reared in the country and to a 20% of the national bovine meat production (ISTAT, 2002). In recent years the number of Veneto beef farms has remarkably decreased (reduction of 9.6%/year; ISTAT, 2002). However, the number of heads farmed has remained constant, showing a progressive shift towards larger fattening units with intensive farming systems. In this situation a correct and careful management has become a very important task in order to preserve both competitiveness and profitability. To this purpose, the control of the management factors affecting animal health could strongly affect not only animal welfare but also the farm profit. The aim of this study was to investigate the main factors related to the state of health of the beefs farmed under intensive breeding systems in the Veneto region.

Material and methods

From 2001 to 2003 health data belonging to 588 fattening groups (batches) for a total of 38723 beefs housed in 29 farms located in the Veneto region were collected. The animals within group of fattening were homogeneous by age and belonged to one of five different genetic types: Charolais (CHA), Female Charolais (FCHA), French Crosses (FC), Limousine (LIM) and Polish Friesian (PF). Data recorded were number of dead and injured

animals, and, for each animal, the type of affection (R: respiratory system; D: digestive disorder; L: locomotor system; N: nervous system; U: urinary apparatus; F: fractures). Data were analysed by logistic regression (SAS, 1990) including the effects of farm (29 levels), genetic type (5 levels), mean batch initial body weight (corrected for the effects of the farm and the genetic type: 1-Light: 311 kg; 2-Medium: 335 kg; 3-Heavy 391 kg), housing system (1: bedding; 2: slatted floor), season (1-Winter: January-March; 2-Spring: April-June; 3-Summer: July-September; 4-Autumn: October-December), year (3 levels) and diet (1-Dry: based mainly on hay; 2-Wet: based on corn silage or pressed sugar beet pulps). The model included also the average daily gain (ADG) and the density (m^2 available/animal) as covariates. Factors were compared using the Wald Chi-Square Test (SAS, 1990).

Results and conclusions

The main causes of injury and death (about 42% of incidence) were problems of the locomotor system and particularly lameness (Figure 1). This is in accord with the results of Murphy *et al.* (1987) that found lameness as one of the most important problems of beefs housed intensively. Cozzi *et al.* (2005) have also noted, in spite of the use of permanent bedding, that about 8% of the bulls considered in their study suffered from lameness. Despite the absence of control in this study, according to Blowey (1993), lameness has resulted to be especially due to environmental and/or nutritional causes. The second main cause of health problems (average incidence of 24.3%) were respiratory problems. The only exception was for FCHA, whose respiratory problems have resulted more important than the locomotor ones (42.6% *vs.* 26.2%). Digestive problems, mainly diagnosed as diarrhoea and ruminal meteorism, showed a general incidence of about 9% and were the third source of health problems in the beefs. Other injury or death causes had little importance (usually <5% incidence). The only exception was for the urinary problems in the FC genetic type, that showed a 12.3% of incidence (Figure 1).

Table 1 shows the comparisons between the relative risks (Odd Ratios) for the analysed factors with a significant incidence. The farm effect had the highest magnitude. This suggests that, despite generally similar management practices, differences in individual practices and management attitude remain of great influence on the percentage of dead and injured animals among different farms. Other important explanatory variables were genetic type, ADG, batch initial body weight, housing system and the density of the animals. Within the genetic type effect, the comparison that showed the lowest significant incidence of health problems was the contrast between FCHA and PF, that showed a 70% higher probability of dying or getting injured than FCHA. This reflects a more

Figure 1. Incidence (overall and within genetic type) of the single health problems in different systems (R: respiratory; D: digestive; L: locomotor; N: nervous; U: urinary) or related to fractures (F).

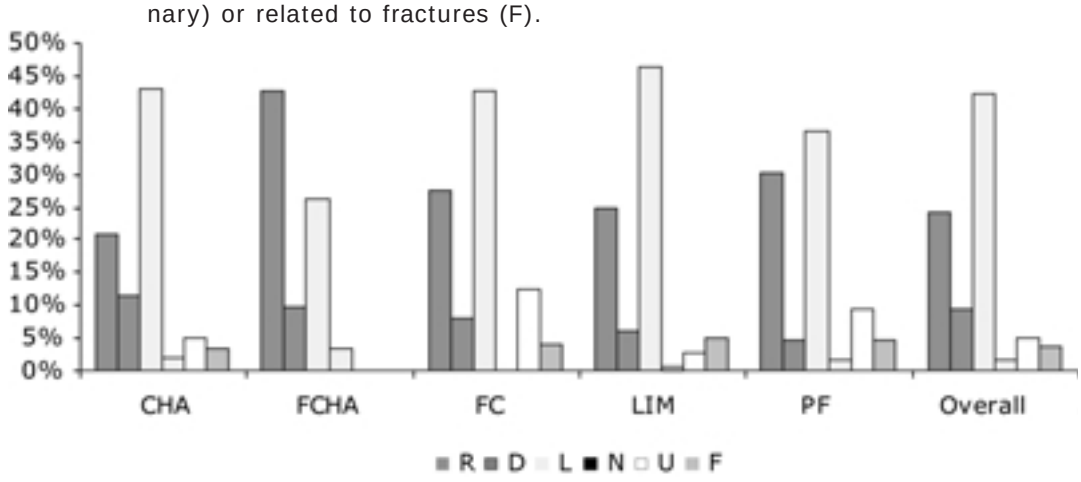

Table 1. Estimate of the odd ratios for the effects with statistical significance.

Comparisons	Odd Ratios	Wald Test Confidence Limits at 95% Minimum	Maximum
CHA *vs.* PF	1.26	0.85	1.87
FC *vs.* PF	0.93	0.62	1.38
LIM *vs.* PF	0.77	0.56	1.06
FCHA *vs.* PF	0.27	0.16	0.46
Bedding *vs.* Slatted floor	0.67	0.53	0.85
Wet *vs.* Dry feeling	0.75	0.38	1.50
Light *vs.* Heavy initial weight	1.34	1.13	1.60
Medium *vs.* Heavy initial weight	1.14	0.95	1.37
ADG	0.15	0.06	0.38
Density	1.10	1.01	1.21

careful control of the environmental conditions and sanitary procedures when FCHA are reared. Indeed, the PF breed, that has a lower market value, was characterised by the highest percentage of dead and injured animals (3.73 for PF *vs.* 2.41% for the other breeds). It is impossible, at this stage of analysis, to distinguish between the effects of farming practices during fattening in the Veneto farms and the residual effects of the previous weaning and rearing period in the Countries of origin. About the housing effect, the use of bedding showed to reduce the risk of culling by 33%. This means that there are 7 eliminated animals on the bedded floor every 10 dead and injured animals on the slatted one. As regards the initial body weight, the lightest weights showed a higher risk of culling (34%) than the heaviest ones, most probably because of the longer productive cycle and therefore of the longer stay in the farm. The probability of dying or getting injured decreased of 8.5% for every 100 g of daily growth above the average value. On the contrary, this probability increases of 10% for every m^2 available/animal over the average density. This result is the only one that is apparently in contradiction with the expectations but it could be explained by the positive relationship existing between space availability and movement possibility, that predisposes the animals to a higher incidence of locomotor problems. Year, season and feeding regimen did not show any relationship with incidence of the health problems. This indicates that the intensive beef production system is constantly affected by a similar incidence of healthy problems in different years and seasons. However, because of the general low incidence of the healthy problems, it is not possible to differentiate within season and year the causes of injury and death. In conclusion, the present study can be considered a guide to face the problem of culling in intensive beef production systems. Particularly, the study indicates that a careful management and especially bedding, length of the fattening cycle, ADG and density are strategic factors for controlling the incidence of health problems in beefs.

Authors are grateful to farmers belonging to beef producer association AZOVE (Associazione Zootecnica Veneta) and to G. Borin, L. Tondello, G. Dalle Rive,. C. Ceccato, E. Florian and P. Lanza for their contribution to the study.

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SHORT COMMUNICATION

Effect of production system on fatty acid composition of meat from Simmental bulls

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ABSTRACT

The objective of the study was to asses the effect of the production system on fatty acids (FA) composition of intramuscular fat of three muscles (*longissimus dorsi, semitendinosus, diaphragmae*) from Simmental bulls. Bulls (n=8) were fattened either with maize silage and concentrates or on pasture. Grazing bulls showed a significant lower percentage of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), but had higher percentage of polyunsaturated fatty acids (PUFA) and conjugated linoleic acid. The n-6/n-3 PUFA ratio was significantly lower (P<0.001) and the PUFA/SFA ratio was significantly higher (P<0.001) in muscles of grazing bulls. The lowest SFA percentage was found in *semitendinosus* (36.5±0.8) followed by *longissimus dorsi* (44.4±0.8) and *diaphragmae* (48.2±0.8) muscle. The percentage of PUFA and n-3 PUFA was the highest in *semitendinosus* muscle, while the n-6/n-3 PUFA ratio was similar in all three analysed muscles.

Key words: Pasture, Cattle, Fatty acids, Muscle

Introduction

Composition of fat in muscle and adipose tissue of farm animals is influenced by different factors such as species, diet, fatness, age or body weight, gender, breed (Nürnberg *et al.*, 1998). Beef meat is an important dietary source of proteins and beneficial fatty acids (FA) for humans, included n-3 polyunsaturated fatty acids (PUFA) and especially conjugated linoleic acid (CLA). In relation to human health, lower ratio of PUFA to saturated fatty acids (SFA) in dietary fat is important. A study of Enser *et al.* (1996) showed that the n-6/n-3 PUFA ratio is lower in ruminant meat in comparison to pork meat. Effects of diet and breeding conditions have been studied, too (Ender *et al.*, 1997, Enser *et al.*, 1998, Mendell *et al.*, 1998, French *et al.*, 2003, Nuernberg *et al.*, 2005). Intensification of meat production caused an increase in concentrate content in the rations of ruminants, which reflected in modified FA composition of produced meat. French *et al.* (2000) demonstrated that when animals were grown at the same rate, muscles from cattle which had a high grass intake had a higher PUFA/SFA ratio and a lower n-6/n-3 PUFA ratio than muscles from cattle fed concentrates. The objective of this study was to asses the effects of fattening system on FA composition in three different muscles in Slovenian Simmental bulls.

Material and methods

Sixteen Slovenian Simmental bulls were used to evaluate the effect of production system on beef FA composition. In maize (C) experimental group, bulls (n=8) were fattened with a maize silage (85% DM) based diet plus a concentrate (soy bean and

Table 1. Fatty acids (% of total weight) composition of muscles (L-*longissimus dorsi*, S-*semitendinosus*, Di-*diaphragmae*) according to diet (C: maize silage plus concentrates; P: pasture).

	C	P	SEM	L	S	Di	SEM	D	M	DxM
C12:0	0.03	0.02	0.01	0.01	0.01	0.06	0.01	*	***	ns
C14:0	1.97	1.60	0.10	1.95	1.24	2.17	0.12	**	***	ns
C16:0	21.48	18.87	0.41	21.80	18.35	20.39	0.51	***	***	ns
C18:0	17.94	18.29	0.37	17.66	14.50	22.19	0.45	ns	***	ns
C18:1	33.46	28.30	0.71	33.51	27.48	31.66	0.86	***	***	ns
C18:2n-6	11.45	14.74	0.69	10.62	18.38	10.28	0.85	**	***	ns
C18:3n-3	0.71	3.71	0.15	1.83	2.93	1.86	0.18	***	***	**
CLA	0.31	0.44	0.02	0.41	0.30	0.42	0.03	***	**	ns
C20:0	0.13	0.12	0.01	0.14	0.10	0.14	0.01	ns	*	ns
C20:3n-6	0.82	0.75	0.07	0.67	1.30	0.39	0.08	ns	***	ns
C20:4n-6	3.80	3.48	0.23	2.86	5.74	2.33	0.28	ns	***	ns
C20:5n-3	0.21	1.06	0.07	0.50	1.03	0.37	0.08	***	***	**
C22:6n-3	0.03	0.12	0.01	0.05	0.11	0.06	0.02	***	*	ns
SFA	44.24	41.81	0.65	44.36	36.52	48.19	0.80	**	***	ns
MUFA	37.23	31.77	0.77	37.21	31.36	34.93	0.94	***	***	ns
PUFA	18.61	26.52	1.16	18.47	32.2	16.95	1.42	***	***	ns
n-3PUFA	1.51	6.58	0.29	3.36	5.80	2.98	0.35	***	***	**
n-6PUFA	16.76	19.24	0.97	14.55	26.15	13.30	1.19	ns	***	ns
PUFA/SFA	0.45	0.68	0.04	0.42	0.91	0.36	0.05	***	***	ns
n-6/n-3	11.01	3.00	0.19	6.98	7.01	7.02	0.23	***	ns	ns

*ns: not significant; *: P<0.05; **: P<0.01; ***: P<0.001*

sunflower meal). C-bulls were fattened 8 months with the described diet and then slaughtered at an average carcass weight of 316 kg, and were scored into fatness class 3.0. Bulls from pasture group (P, n=8) were grazed on pasture from April till October, without any additional supplements. At slaughter (end of October) they showed a mean carcass weight of 371 kg, and fatness score of 2.8. Bulls from group P were heavier at slaughter, but had comparable degree of fatness with group C. Bulls from both diet groups were slaughtered younger than 2 years. Samples of *longissimus dorsi* (L), *semitendinosus* (S) and *diaphragmae* (D) muscles were vacuum-packed, rapidly frozen and stored at -70°C until analysis of FA composition. On the basis of previous work (Stibilj and Koman Rajšp, 1997) and due to simplicity, speed and reduced organic solvent usage, we omitted lipid extraction of the tissue samples and performed *in situ* transesterification (ISTE) of fatty acids (Park and Goins, 1994). Methyl esters of fatty acids (FAMEs) were analysed by gas chromatography (Agilent 6890 Series GC System equipped with Agilent 7683 Series Injector and Agilent 7683 Series AutoSampler). Separation of FAMEs was performed on capillary column Omegawax™ 320 (30 m x 0.32 mm x 0.25 µm film thickness). Individual FAMEs were identified by comparison of retention times with those of standard mixtures and quantified using response factors derived from quantitative standards (NuCheck). The results are given as % of total weight of fatty acids. Data were statistically analysed with General Linear Model (GLM) procedures with fixed effects of diet, muscle type and their interaction (SAS, 1999).

Results and conclusions

SHORT COMMUNICATION

Factors affecting growth performance in beef production: an on farm survey

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ABSTRACT

This study aimed to investigate sources of variation of average daily gain in beef cattle, with an on farm approach, in the Veneto region. Data of 38707 animals fattened in 29 farms and belonging to 5 genotypes (Charolais, Charolais Female, French Crossbred, Limousin and Polish Friesian) were analysed considering type of farm, farm nested within type, genotype, housing system, arrival live weight, percentage of dead and injured animals, season of sale and diet. Genotype, farm, percentage of unhealthy animals and season of sale significantly affected average daily gain. Charolais and French Crossbred showed the highest values of average daily gain, Charolais Female the lowest. The effect of farm suggests that management is very important for growth performance in beef cattle. Moreover, batches of animals with high percentage of dead and injured presented lower values of average daily gain. In spring-summer 2003 an extremely hot climate was unfavourable for growth performance.

Key words: Growth performance, Beef production, Average daily gain, On farm survey

Introduction

Growth performance is a characteristic of relevant economic importance in beef cattle production system, one of the most developed agricultural sector of UE. A number of authors studied growth performance in beef cattle, and in particular daily gain (Schwartzkopf Genswein *et al.*, 2004; Bruns *et al.*, 2005). In general these researches are based on experimental plans, and only few considered on farm surveys. This study aimed to investigate variability and sources of variation of daily gain in some beef cattle genotypes widespread in Veneto region, with an on farm approach.

Material and methods

This research involved an analysis of growth performance for 5 genotypes (Charolais, Charolais Female, French Crossbred, Limousin and Polish Friesian) of beef cattle fattened in the Veneto region. Data were collected in 29 farms associated to AZOVe (Associazione Zootecnica Veneta), and included information about characteristics of the farm and of the batch of animals, growth performance during fattening, sanitary and nutritional aspects. Homogeneous batches of animals were identified by genotype, farm, fattening period and characteristics of the diet. Every single batch was the reference unit for descriptive and statistical analysis. Final database included 583 batches for a total of 38707 animals, fattened from 2000 to 2003.

Data were analysed by using the SAS package (1990). Continuous variables included in statistical analysis were classified in fixed effects: percentage of dead and injured animals was grouped in three classes (low, moderate, high) on the base of standard deviation by genotype; arrival live

Table 1. Descriptive statistics for growth performances by genotype (583 batches).

Genotype	No. batches	Average weight at arrival (kg)		Final average weight (kg)		Average daily gain (kg/d)	
		mean	SD	mean	SD	mean	SD
Charolais	253	385.1	48.4	675.4	30.6	1.37	0.10
Charolais Female	109	303.1	29.1	494.1	23.6	0.95	0.08
French Crossbred	43	374.1	37.3	646.3	26.6	1.28	0.10
Limousin	153	305.4	39.3	580.0	32.9	1.20	0.09
Polish Friesian	25	283.1	23.1	539.4	33.4	1.16	0.06

weight was classified in three classes according to the same procedure; space for animal was classified in large (>4 m^2 for bedding, >3 m^2 for slatted floor) and small and combined with the effect of type of housing system (bedding and slatted floor). Average daily gain (ADG) was analysed by ANOVA according to a linear model which included the fixed effects of the type of farm (cooperative, private with more than 500 places, private with 150-500 places, private with less than 150 places), farm nested within type, genotype, interaction between housing system and space for animal, class of arrival live weight, class of unhealthy animals and season of sale.

Detailed information about diet was available for a sub-sample of 268 batches, for which percentage of starch, protein, ensiled pressed sugar beet pulps, and corn silage of the diet were known. These variables were included in the general linear model as covariates (corrected for genotype) with the aim to investigate the effects of characteristics of the diet on the ADG of considered batches of animals.

Results and conclusions

In Table 1 descriptive statistics for growth performance for each genotype are reported. Charolais was the most widespread genotype, whereas French Crossbred and Polish Friesian were the genotypes with the lowest number of batches farmed. Average weight at the arrival ranged from 283 (Polish Friesian) to 385 kg (Charolais) with a moderate variability between batches of the same genotype. Charolais and French Crossbred showed the highest values of final average weight, Charolais Female were more light than the other genotypes. ADG presented the same trend, with higher values for Charolais and French Crossbred and lower values for Charolais Female.

Table 2. Sources of variation for ADG (583 batches).

Source of variation	DF	Mean square	F	P
Type of farm	3	0.007	1.14	n.s.
Farm (type of farm)	25	0.038	6.20	***
Genotype	4	1.057	171.51	***
Housing system x space	3	0.009	1.42	n.s.
Class of arrival live weight	2	0.004	0.58	n.s.
Class of unhealthy	2	0.023	3.73	*
Season of sale	11	0.026	4.26	***
R^2(%)		83.3		

*n.s. = not significant, *** P<0.001, * P<0.05*

Table 3. Descriptive statistics for growth performances by genotype (583 batches).

Genotype	No.	Daily dry matter intake (kg /100 kg)		Starch (% of DM)		Protein (% of DM)		Corn silage (% of DM)		Pulps (% of DM)	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Charolais	109	1.75	0.12	29.5	2.2	14.3	0.6	8.0	2.1	4.7	2.2
Charolais Female	46	1.91	0.10	16.0	5.6	16.0	0.3	5.0	2.6	9.6	3.6
French Crossbred	20	1.76	0.16	28.7	1.8	14.2	0.7	8.1	1.6	2.6	2.1
Limousin	80	1.79	0.14	29.4	2.4	14.4	0.7	5.0	2.8	3.1	1.9
Polish Friesian	13	2.01	0.12	24.7	5.1	14.0	0.7	7.4	3.4	5.0	4.2

In Table 2 results of ANOVA performed for ADG are reported. The model had a very high coefficient of determination (83.3%). Farm nested within type, genotype, class of unhealthy and season of sale significantly affected ADG, whereas type of farm, class of arrival live weight and interaction between housing system and space were not significant.

Statistical significance of type of farm effect was tested using the mean square of farm nested within type as error term, and this test confirmed that type of farm did not affect significantly ADG. The strong effect of genotype was expected and it confirms the descriptive statistics reported in Table 1, with high values of ADG for Charolais and French Crossbred, intermediate for Limousin and Polish Friesian and low for Charolais Female (least square means, data not shown in table). The statistically significant effect of farm suggests that management has an important role on variability of growth performance. ADG was lower for batches with a high percentage of unhealthy (dead and injured) animals with respect to batches with low or moderate percentages (Figure 1).

In Figure 2 least square means for season of sale on ADG are presented. ADG showed a moderate variability between seasons of different years, except for 2003 when the values of ADG were significantly lower than for the other years, especially in Spring, Summer and Autumn. It can be due to the extremely hot climate in that year, unfavourable for growth performance of the animals.

Characteristics of the diets were similar for Charolais, French Crossbred and Limousin, which differed from Charolais Female and Polish Friesian (Table 3). Within genotype, variability was generally low, indicating a homogeneity of feeding standards within AZOVe. The ANOVA performed for this sub-sample did not show any significant effect of diet on ADG (data not shown in table).

These results confirm that ADG is strongly related to genotype, and that, with the range of variability used in practice, feeding and housing have a minor importance. The fact that performances remain highly variable within farms and are affected by percentage of unhealthy animals indicated that strategies to improve performances should aim primarily to identify gaps in management practices. Also climate (summer high tem-

Figure 1. LS means for unhealthy on ADG.

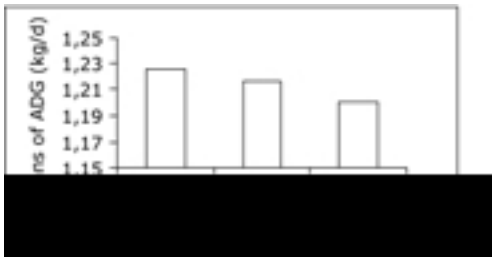
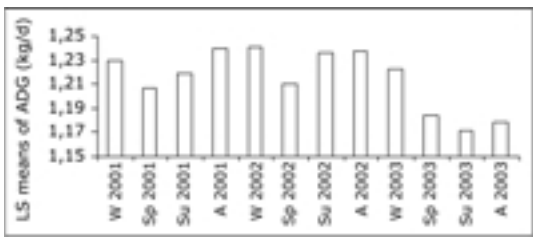


Figure 2. LS means for season of sale on ADG.



peratures) appears to be a potential limiting factors. The inclusion in this analysis of other batches of animals should provide further insight into the relationships between farm system and growth performance of beef cattle in different genotypes.

Authors are grateful to G. Borin, L. Tondello, G. Dalle Rive, C. Ceccato, E. Florian and P. Lanza of AZOVe (Associazione Zootecnica Veneta) for their support to the research.

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SHORT COMMUNICATION

Effectiveness of different strategies to prevent from heat stress in a group of dairy farms located in the Province of Padova

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ABSTRACT

Heat stress during the summer season is an important factor which can impair dairy cows physiology and productivity. A survey was carried out on a sample of 30 dairy farms of the Province of Padova to assess the effectiveness of different strategies for heat stress control. All farms used a fan cooling system but in those were a sprinkler device was also operating an increased milk yield was observed (+5.0%). Cows receiving the diet in two daily distributions (morning and evening) increased DM intake (+9.0%) and milk yield (+15.0%) in comparison to animals fed once a day. No difference, instead, were observed in farms where cows were fed once a day in the morning or in the evening. A positive milk response (+8.1%) was recorded in farms equipped with wide waterers at the exit of the milking parlour.

Key words: Dairy cow, Heat Stress, Cooling devices, Feeding management

Introduction

Heat stress is the most important factor impairing dairy cows productivity and fertility during summer months (West, 2003). A wide number of studies have been carried out with dairy cattle to select the best solutions capable to limit the detrimental effects of thermal stress. The installation of cooling devices has always been recommended and the association of fans and sprinklers has been reported to increase feed intake (+9.2%) and milk yield (+15.9%) (Turner *et al.*, 1992). The improvement of watering system is also recommended to compensate the greater water losses due to the activation of thermoregulatory mechanisms by the animals (Shalit *et al.*, 1991). Other management strategies, such as the evening administration of TMR, have be suggested in order to stimulate DMI (Aharoni *et al.*, 2004). The aim of the present study was to evaluate the effectiveness of some of these solutions in a representative sample of dairy farms of the Province of Padova during the summer season of the year 2004.

Material and methods

The study used data collected in 30 dairy farms located in the Province of Padova. All farms raised Italian Holstein cattle and the average herd size was 73 ± 36 cows. The lactating cows were housed in free stall barns in which a fan cooling system was operating during the summer season. Fan diameter was lower than 130 cm in 14 cases while in the remaining 16 it was greater than 130 cm. In addition to the fans, sprinklers were oper-

evaluated the effect of the waterers outside the milking parlour. All the three models included also the effects of farm and, as covariate, the herds mature cow equivalent and days in milk available at the time of the 2 visits.

The diameter of the fans operating in the barn did not affect THI as well as cows intake, physiologic parameters, and milk response (Table 1). Also the sprinklers did not improve the environmental conditions of the barn along with the values of several heat stress indicators measured on cows (Table 1). However, it must be pointed out that since the sprinklers were turned on manually in all farms, most of them were not working at the time of data collection and this could have biased the resuluiMve thdata *-0. A2d

Table 2. Effect of the time and number of daily distribution of the diet on feed intake and milk production.

		Time of diet distribution			RMSE
		Morning	Evening	Morning & Evening	
Herds	Number	17	7	6	
DM intake	kg	20.6^{b}	21.6^{ab}	23.0^{a}	1.6
Milk yield	kg	27.8^{β}	27.3^{β}	31.6^{α}	2.4
Milk fat	%	3.61	3.66	3.61	0.15
Milk protein	%	3.25	3.19	3.29	0.09

Means within row with different superscript (a, b) differ at P <0.05 and (α, β) differ at P <0.10

TMR while they are less motivated to eat a diet which remains in the manger for a long time. Consistent with the highest intake, cows fed twice a day showed an increased milk production with no difference in milk composition (Table 2).

The presence of waterers longer than 100 cm at the exit of milking parlour had a positive effect on DM intake and milk yield (Table 3). On the contrary, when the size of the waterers was below 100 cm the performance of the animals were similar to those recorded in the farms without any water provision outside the milking parlour. Dairy cows behaviour could explain these results. The reduced space at the water trough as well as at the manger, can affect cattle social behaviour, increasing competition among animals. In this situation it is likely that the low ranking subjects of the herd will be penalized in their freedom to visit these areas (Syme and Syme,1979).

Considering the different strategies for the heat stress control evaluated in the present study we can conclude that the fan's size seems to have a minor influence on dairy cows physiology and productive response. It is likely that the number and location of the fans within the barn will play a more important role than their size. Cows have shown to benefit from the use of sprinklers, however the effectiveness of this cooling device could be maximized by the adoption of an automatic turn-on system capable to start the cooling device when THI inside the barn reaches the critical threshold of 75. Based on cows intake and productive response, it seems recommended to split in two distribution the daily TMR administration during the summer season, while the single evening delivery has not shown any advantage in comparison to the morning one. Finally, the survey has demonstrated the role played by the water

Table 3. Effect of presence and size of the water trough located outside the milking parlour on feed intake and milk production.

		Water trough size outside the milking parlour			RMSE
		None	< 100 cm	> 100 cm	
Herds	Number	5	10	15	
DM intake	kg	20.1^{β}	$21.0^{\alpha\beta}$	21.6^{α}	1.6
Milk yield	kg	27.2^{b}	27.4^{b}	29.5^{a}	2.3
Milk fat	%	3.56	3.67	3.63	0.14
Milk protein	%	3.32	3.24	3.25	0.10

Means within row with different superscript (a, b) differ at P <0.05 and (α, β) differ at P <0.10

provision at exit of the milking parlour. It is advisable to adopt big size waterers capable to allow the simultaneous visit of all the cows exiting together after the milking. However, in order to prevent the heat stress, the provision of drinking water outside the milking parlour must not replace the maximum availability of water in the other housing areas of the barn.

Authors are grateful to the extension service personnel of the Provincial Farmer Association (A.P.A.) of Padova for its precious technical support in data collection and processing.

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SESSION I

FREE COMMUNICATIONS

FREE COMMUNICATION

Relation between endosperm and *in vitro* kinetic of starch digestibility in maize hybrids for broiler chickens

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ABSTRACT

The objective of this study was to determine relationship between endosperm type of maize hybrids produced in Croatia and kinetic of starch digestibility provided by *in vitro* method. Proportion of endosperm type (horny and floury) were used to classify hybrids into dents or flints maize. Flints had less rapidly digestible starch than dents. Maize hybrid kernel endosperm characteristics could be used for prediction of digestibility kinetics of maize grain.

Key words: Maize, Endosperm, Starch, Digestibility

Introduction

Maize has been considered to have fairly constant energetic value. Though, there is growing evidence today that the feeding value of maize samples can vary significantly based on animal performance. Still there is no data available to suggest the reasons for these divergences in energetic value. Variability in composition and quality affects the metabolizable energy content with consequential effects on broiler performance (D'Alfonso, 2003). Maize starch provides more than 50% of apparent metabolizable energy (AME_N) of common diets for broiler chicken in Croatia and any factor which has an effect on starch digestibility would be expected to influence AME_N in maize based broiler diets. Maize starch is almost entirely (97-99%) contained in endosperm. Flint maize has hornier and dent maize has flourier endosperm. Rose et al. (2001) found significant correlation between both weight gain and feed conversion ratio and the wheat grain hardness. Weurding (2002) and D'Alfonso (2002) found that the starch of similar total digestibility give different broiler chickens performances. Weurding *et al.*, (2002) showed that site, rate and extent of starch digestion in small intestine of broiler chickens differ considerably within a wide range of untreated feedstuffs. Broiler chickens grow faster and more efficiently on a diet containing slowly digestible starch than on a diet containing rapidly digestible starch (Weurding *et al.*, 2003). There is a lack of information about the relation between the kinetics of maize starch digestion and type of endosperm. The objective of present study was to investigate differences in the site of starch digestion between some maize hybrids in Croatia.

Material and methods

This study was performed with twelve maize hybrids (Bc 354, Bc 462, Bc 4982, Bc 566,Bc 5982, OSSK 303, OSSK 444, OSSK 373, Stefania,

Table 1. Influence of endosperm type on maize grain characteristics and *in vitro* kinetics of starch digestion.

Hybrid	Type	Horny	Flory	Sugar, % DM	Starch, % DM	RDS	SDS	RS
OSSK303	F	85.16	14.85	0.49	71.53	65.52	22.99	11.49
BC462	F	79.27	25.73	0.74	73.60	66.80	22.43	10.77
GALICE	F	73.79	22.38	1.11	72.12	71.95	21.28	6.78
STEFANIJA	F	71.97	28.04	1.00	71.78	69.44	23.51	7.05
OSSK373	F	71.82	28.18	0.86	71.06	72.89	21.63	5.48
BC6661	F	70.48	29.55	0.42	85.48	70.82	21.44	7.74
BC566	D	67.98	32.02	0.90	73.83	73.82	18.17	8.01
BC4982	D	66.85	33.15	0.89	69.83	71.49	21.85	6.65
OSSK444	D	65.75	34.25	0.81	69.93	71.90	23.43	4.67
F70	D	63.79	36.21	1.12	71.10	73.14	23.38	3.49
FLORENCIA	D	62.60	37.41	0.95	74.30	72.03	20.77	7.20
BC5982	D	58.28	41.73	0.50	87.46	75.24	18.55	6.22
Mean		69.81	30.29	0.82	74.33	71.25	21.62	7.13
SEM		2.13	2.09	0.07	1.69	0.81	0.51	0.65
P-value		P<0.01	P<0.01	ns	ns	P<0.05	ns	ns

Florencia, F70, Galice) differing in the proportion of horny and floury endosperm. According to Philippeau and Michalet-Doreau (1998) the (semi)dent maize (D) is characterised by the presence of a horny endosperm at the side and back of the kernel; the central core extends to the crown of the kernel and is floury. (Semi)flint (F) maize has a horny endosperm surrounding a small proportion of the floury endosperm. A kernel of flint maize is rounded with no denting. The mass content of both horny and floury endosperm was determined by manual dissection of the kernel (Correa *et al.*, 2002). *In vitro* starch digestion procedure simulates the consecutive digestion processes in various parts of the broiler alimentary tract. Briefly, in Weurding (2002) procedure test tubes containing the maize samples, glass balls, a mixture of digestive enzyme and buffer solution were incubated in a shaking water bath (37°C). After nine incubation times (0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5 and 6 h) aliquots were taken from the tubes and the amount of released glucose was measured colorimetricaly. Starch digestion coefficients were calculated for each incubation time and were used for further estimation of the proportion of each starch fractions digestibility. Rapidly digestible starch (RDS) represents starch digestion after 2 h incubation whereas slowly digestible starch (SDS) represents digestion after 4 h, while resistant starch (RS) is not digestible in small intestine. Dry matter (105°C, 16h), starch with enzymatic method (Englyst *et al.*, 1992) and sugar colorimetric were determined. All analysis were performed in duplicate. Differences *in vitro* kinetics of starch digestion between maize hybrid types were tested by means of the Student t-test (SAS, 1987).

Results and conclusions

The characteristics of the 12 maize hybrids are described in Table 1. The horny endosperm ranged in wide range (58.28 to 85.16%) among maize hybrids, and averaged 75.41 and 64.21% in flint and dent types receptively. The average content of both starch (74.41 *vs.* 74.26%) as well as sugar (0.86 *vs.* 0.75) were similar (p>0.01) for the dent and flint types. The flint and dent maize markedly differed (P<0.05) in RDS. According to

Table 2. Coefficient of correlation between maize endosperm type and *in vitro* kinetics of starch digestion.

Variable	Kinetics of starch digestion		
	RDS	SDS	RS
Horny endosperm	-0.87**	0.42	0.75**
Floury endosperm	0.78**	-0.39	-0.66*
Starch % in DM	0.29	-0.55	0.07
Sugar % in DM	0.34	0.09	-0.38

** p<0.05, ** p<0.01*

McAllister *et al.* (1993) starch is embedded within protein matrix and shielded by cell walls in flint maize and these physical structures may adversely effect starch digestion in the small intestine of broiler chickens. All maize hybrids had similar and uniform level of slow digestible starch, while flint maize had higher resistant starch (p>0.01) due to wide variation (3.48-11.49%) between maize hybrids.

The endosperm characteristics had strong affect on the kinetics of in vitro maize starch digestion (Table 2). The horny endosperm content was negatively (-0.87; P<0.01) correlated with the rate of starch digestion, and it could be an accurate predictor of rate of starch digestion for maize kernels. Philippeau and Michalet-Doreau (1998) observed a value of 88.5% variation in ruminal starch digestion was associated with virtuousness. Weurding et al (2003) showed that birds fed with relatively more slowly and resistant starch consume more feed and grow faster. Grbesa et al (2003) found that chickens fed with flint maize hybrids have larger weight of the age of 21 days. The proportion of endosperm type varies considerably between examined maize hybrids. The flint maize had less rapidly digestible fraction of maize starch in the small intestine of chickens than dent maize hybrids. Kernel endosperm characteristics may be an accurate predictor of rate of starch digestion for maize kernels.

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FREE COMMUNICATION

Estimating grass and grass silage degradation characteristics by *in situ* and *in vitro* gas production methods

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ABSTRACT

Fermentation characteristics of grass and grass silage at different maturities were studied using *in situ* and *in vitro* gas production methods. *In situ* data determined difference between grass and silage. Degradable fraction decreased as grass matured while the undegradable fraction increased. Rate of degradation (kd) was slower for silage than fresh grass. Gas production method (GP) data showed that fermentation of degradable fraction was different between stage of maturity in both grass and silage. Other data did not show any difference with the exception for the rate of GP of soluble and undegradable fraction. The *in situ* degradation characteristics were estimated from GP characteristics. The degradable and undegradable fractions could be estimated by multiple relationships. Using the three-phases model for gas production kd and fermentable organic matter could be estimated from the same parameters. The only *in situ* parameter that could not be estimated with GP parameters was the soluble fraction. The GP method and the three phases model provided to be an alternative to the *in situ* method for animal feed evaluations.

Key words: Grass, *In situ*, Gas production, Maturity

Introduction

Dynamic of nutrients digestion in the reticulorumen is one major determinant of utilization of feedstuffs by ruminants. Rate, extent, and synchronization of carbohydrates and protein degradation in feedstuffs can affect the rumen fermentation pattern, organic matter digestibility, and microbial protein synthesis and rumen health. Plant maturity and ensiling are some of the most important factors affecting the rate and extent of digestion of food components within the rumen and essential in predicting the nutritive value of grasses.

Incubation of feedstuffs at different time and a measure of *in situ* kinetic of rumen degradation (Mehrez and Ørskov, 1977) is used as basis in many feed evaluation systems. Since the late 170s, measurement of in-vitro gas production (GP) has become increasingly popular for determining forage digestion characteristics and the kinetics of fermentation. Scientists are using classical gas production method described by Menke *et al.* (1979) and its modifications that are automated and use different electronics valves, pressure transducers and computers. The purpose of those investigations is the improvement of accuracy and the reduction of time for each sample, i.e. greater number of feed samples can be processed at any one time (Davies *et al.* 2000). The GP method is used as a research instrument to study fermentation characteristics of ruminant food, in breeding programs of grass and to search optimal degradable genotypes. Cone *et al.* (2002) showed a

Table 1. Fermentation characteristics of grass and silage samples, in situ method.

Sample	Maturity	S	D	U	k_d	FOM
grass	1	24,64	61,61	14,75	7,27	76,51
	2	25,65	59,69	15,66	7,27	76,05
	3	31,41	53,43	16,16	4,85	76,26
	4	23,63	48,28	29,09	6,06	65,27
	5	23,84	49,19	27,98	5,45	66,24
	6	21,92	46,46	32,62	4,85	62,32
silage	1	30,30	55,35	15,35	4,65	74,03
	2	34,44	51,31	15,25	4,34	74,55
	3	27,98	50,10	22,93	4,65	68,97
	4	36,06	42,42	22,52	3,74	70,33
	5	31,01	46,16	23,84	3,43	68,83
	6	28,08	48,38	24,54	3,03	68,63
	SEM	1,30	1,63	1,80	0,39	1,38
Sample significance		*	ns	ns	**	ns
Maturity significance		ns	**	**	ns	**

close relationship between in situ degradation characteristics and GP parameters for concentrate feedstuffs. The aim of this study is to investigate the possible relationship between *in situ* degradation characteristics of OM and GP characteristics in grass samples that differ in maturity and type of conservation (fresh and ensilaged).

Material and methods

The study was perform with grass *Lolium perenne L.* harvested at six stage of maturity. Grasses at different stage of maturity were harvested every seven days after first cut. All of the determination on samples, six grasses and six grass silages were performed two times in triplicate. Grass (approximately 45% DM) was ensiled for six week in 30 l laboratory silos. Rumen degradability of grass and silages was measured by the in situ procedure of Mehrez and Ørskov (1977). The soluble fraction (*S*) was determined by washing with cold tap water in a washing machine for 45 min. The residue after 336 h (14 days) incubation in the rumen fluid was considered undegradable fraction (*U*). The degradable fraction (*D*) was calculated as 100-S-U. Data was fitted to a first-order degradation model (Robinson *et al.*, 1986). Content of fermentable organic matter (FOM) was calculated from *in situ* incubations. Determination of GP of was carried out with a fully automated system as described by Cone *et al.* (1996). Curves of GP were fitted with a three-phase model, as described by Cone *et al.* (1996) and Groot *et al.* (1996). The non-linear model of the SAS package (SAS, 1989), was used to determine in situ and in vitro gas production parameters. Correlation between GP and in situ degradability parameters were obtained by the CORR procedure of SAS (1989).

Results and conclusions

In situ data (Table 1) for S ranged from 22% to 36% with no effect ($P \geq 0.05$) of grass maturity, but difference ($P \leq 0.05$) was determined between grass and silage. As grass matured D decreased ($P \leq 0.01$) while the U increased ($P \leq 0.01$) and ranged from 14.8% for the youngest plants to 32.6% for the oldest grasses ($r=0.86$). Rate of degradation (kd) decreased from fresh to ensiled($P<0.05$). Data of GP (Table 2) showed differences between stages of maturity for the fermentation of degradable fraction. Other data did not show difference ($P \geq 0.05$) with exception for rate of gas production. The *in situ* degradation characteristics were estimated from GP parameters. For multiple relationships,

Table 2. Fermentation characteristics of grass and silage samples gas production methods.

Sample	Maturity	a1	b1	c1	a2	b2	c2	a3	b3	c3
grass	1	77,77	3,13	0,90	125,24	7,37	2,75	52,52	33,33	6,16
	2	90,90	4,34	0,77	151,50	8,08	2,28	48,48	37,37	5,56
	3	104,03	2,63	1,05	117,16	8,89	2,44	38,38	28,28	4,95
	4	94,94	2,83	0,90	165,64	10,20	1,80	39,39	41,41	4,04
	5	98,98	2,02	0,84	160,59	11,01	1,78	41,41	40,40	5,25
	6	91,91	2,02	0,78	160,59	13,64	1,69	31,31	38,38	5,35
silage	1	80,80	4,85	1,25	109,08	9,29	2,89	51,51	32,32	5,86
	2	104,03	4,14	1,05	128,27	8,79	2,12	35,35	32,32	6,26
	3	108,07	3,43	0,78	144,43	10,91	2,31	44,44	38,38	5,86
	4	96,96	3,03	0,82	147,46	11,41	1,86	33,33	37,37	7,37
	5	101,00	3,54	0,80	143,42	13,43	2,08	50,50	40,40	4,65
	6	104,03	3,54	0,84	136,35	14,75	1,99	42,42	38,38	5,56
	SEM	2,73	0,25	0,04	5,20	0,68	0,11	2,08	1,17	0,25
Sample signific.	Ns	ns	ns	ns	ns	ns	ns	ns	ns	
Maturity signific.	Ns	*	ns	*	***	**	ns	*	ns	

a_n - is maximum gas production in ml; b_n - time at which half of the maximum gas production (a) is reached in h; c_n - parameter determine the shape of the curve.

independent variables were included in the model with ($P \leq 0.05$). The D and U fractions could only be estimated by multiple relationships ($R^2 = 0.77–0.86$). Using the three-phases model for GP, both kd and FOM could be estimated from the same parameters and the relationships were ($R^2 = 0.86–0.92$). Only S of *in situ* could not be estimated with GP parameters ($p \geq 0.05$).

Maturity of grass and silage caused a decreased degradability. The GP and the three phases model provided to be an alternative to the *in situ* method. *In situ* method is time consuming and impose a greater stress to animals than GP methods that need further investigations to become standard method for animal feed evaluations.

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ABSTRACT

The objective of this study was to evaluate the use of Near Infrared Spectroscopy (NIRS) to analyze maize silage with a portable instrument. The instrument was a Zeiss Corona 45 working between 960 and 1700 nm which was used in Italy, Czech Republic and Poland. Best prediction performances were obtained using the Italian data set. Prediction error were 1.0, 0.16 and 0.4 respectively for DM, CP and NDF on a as is basis. With the instrument from Poland and Czech Republic there were lower accuracy of prediction compared to the Italian dataset, probably for their limited (less than 100 samples) calibration data set. Merging all the data set improved prediction accuracy for CP but not DM. It would appear that some form of instrument standardization is needed before merging data set.

Key words: NIR, Portable instrument, Maize silage

Introduction

In order to ensure maximum production and animal health, diets must be optimized to meet animal requirements (Stone, 2003). The process

Table 1. Regression statistics of the calibration data set.

Constituent	N	Mean	SD	SEC[1]	RSQC[2]	SECV[3]	RSQCV[4]
DM, %	330	32.20	2.79	0.87	0.90	0.97	0.88
CP, % As Is	329	2.12	0.24	0.11	0.79	0.12	0.76
NDF, % As Is	327	15.23	1.12	0.36	0.90	0.40	0.87

[1] SEC= Standard error of calibration; [2] RSQC= R square of calibration; [3] SECV= standard error of cross validation; [4] RSQCV= R square of cross validation.

array instrument for the analysis of maize silage without any sample preparation.

Material and methods

Samples (num=388) of maize silage were collected from Italian cattle farm from the year 2000 until 2004. The samples were stored at -20°C until analysis. Sample were thawed overnight and allow to reach at least 10°C. They were scanned on Zeiss Corona 45 (Helma Italia, Milano) equipped with a turnstep accessory, placing the samples on a large (diameter 180mm) Petri dish that allows scanning over large sampling area. Spectral data were collected between 960 and 1700nm. Scanning time (integration time) was approximately 20ms and each scan lasted for 10s with the acquisition of about 500 scan per samples that were averaged to obtain one spectrum per sample. Samples were then dried at 60°C for 48 h, then ground with a hammer mill fitted with a 1mm screen. Laboratory determination included residual DM (105°C), NDF (Mertens, 2002) and CP. Chemical and spectral data were combined into a file. In order to evaluate performances, 48 samples were kept for validation and the remaining samples (num=340) were used for calibration development. Original spectra were interpolated every 2nm and prediction equations were developed with modified partial least square (MPLS) regression method using WinISI 1.5 (Infrasoft International, USA), with math treatments that included standard normal variate and detrening for scatter correction, first derivative calculated over 4 data point. Evaluation of performances were based on the standard error (SE) of calibration, SE of cross validation and on the validation file on the basis of SE of prediction and bias.

Results and conclusions

The data set comprised a large variability due to sampling over a large geographic area of Italy and the inclusion different growing years. Dry matter averaged 32.3% (SD=2.9), CP 2.1% As Is (SD=0.25), NDF 15.2% As Is (SD=1.2). The validation set had very similar chemical composition to calibration data set.

Estimated prediction errors as indicated by the SE of cross validation (SECV) were small and indicated good accuracy of prediction (Table 1). For DM and NDF, R2 of cross validation (RSQCV)

Table 2. Performance of the calibration equation used to predict a validation data set.

Constituent	N	Mean	SD	SEP[1]	Bias[2]	RSQ[3]
DM, %	49	32.82	2.86	1.05	0.11	0.86
CP, % As Is	49	2.16	0.26	0.14	0.01	0.71
NDF, % As Is	49	15.50	1.21	0.52	0.08	0.82

[1] SEP= Standard error of prediction; [2] Bias= Average difference between reference and predicted values; [3] RSQ= R square.

Figure 1. NIR prediction *vs* Reference method (LAB) for DM in validation.

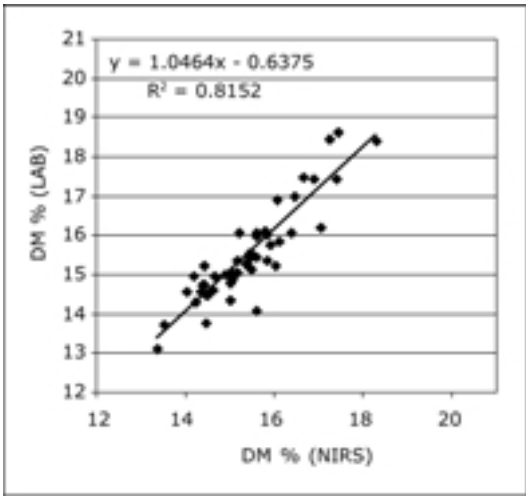
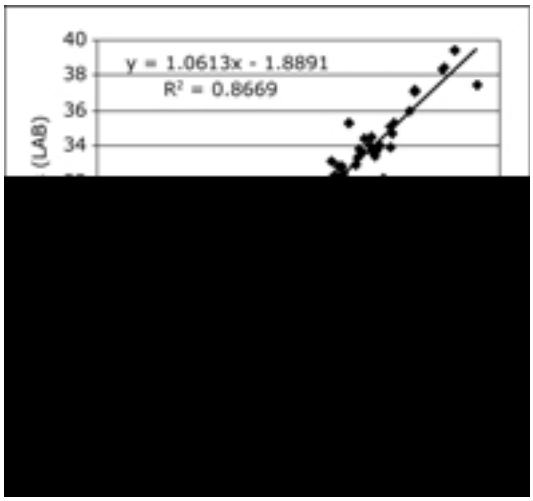


Figure 2. NIR prediction *vs* Reference method (LAB) for NDF in validation.



were around 0.87-0.88. Despite the smallest SECV CP had a RSQCV below 0.80, but this is due to the limited variability of this constituent in maize silage.

The performance of the calibration equation were confirmed in the validation test predicting samples not included in the calibration data set. The SEP values for all three constituents (Table 2) were slightly greater than the SECV of Table 1, but practically very similar. The level of accuracy is practically identical to what reported by Park (1998) on undried unground grass silage that used a laboratory NIR instrument, and similar to prediction accuracy of maize silage predicted, as it is common in NIR, on dried ground samples (Boever, 1997) . The calibration with this portable diode array has proofed to be accurate over the entire range of variation of the data set. The good accuracy along with the competitive cost of the instruments based on diode array can allow to analyze maize silage without any sample processing (drying and grinding) and extent the application of NIR analysis bringing the analytical process directly at farm level.

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FREE COMMUNICATION

Influence of different quantities of rapeseed meal and calcium on production results of broiler chicks

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ABSTRACT

The aim of this research was to establish the influence of different quantities of rapeseed meal (RM) and calcium in feed mix on productive performance of 360 chicks divided in 12 groups during the 42-day experiment. It was found that chicks in group C (0% of RM) and E_{3-5} (10% of RM) achieved significantly ($P<0.05$) higher body gains than those in groups E_{6-11} (20 and 30% of RM) at the end of the experiment. In the same time average body weights of groups $E_{C,3,6,9}$ (0.8% of calcium) were significantly ($P<0.05$) higher than those in groups $E_{1,4,7,10}$ (1.0% of calcium) and groups $E_{2,5,8,11}$ (1.2% of calcium). We can conclude that 10% of RM in broiler diet didn't have a negative effect on the production results. More than 0.8% of Ca in the broiler diet caused lower live weight.

Key words: Broiler chicks, Rapeseed meal, Calcium, Production results

Introduction

As a by-product of the production of rapeseed oil, the most used raw material in production of biodiesel remains a large amount of rapeseed meal (RM). In the order of the consumption of feedstuffs with high protein content, RM is after the soybean products on the second place. After reducing the glucosinolate content of rapeseed by plant breeding, the high content (13.5%) of crude fibre (Mawson *et al.*, 1993) proved to be a limiting factor in the application of greater quantities of rapeseed meal in chicken feed. Researches undertaken indicate that good results can be achieved in broiler rearing if birds are fed diets containing 10 to 15% of rapeseed meal (Jamroz, 1995; Zeb *et al.*, 2002). Feeding of broiler chickens with 1.2% Ca in feed mix didn't have negative effect on body weight and feed conversion (Bakalli *et al.*, 1996). Our preliminary results indicated synergistic effect of higher content of calcium and RM in the broiler diet. Therefore, the aim of this research was to establish the influence of different quantities (10, 20 and 30%) of rapeseed meal (RM) and calcium (1.0, 1.2 and 1.5% in starter and 0.8, 1.0 and 1.2% in finisher diets) on production results of chicks during the 42-day experiment.

Material and methods

The experiment involved 360 Ross broiler chicks divided into twelve groups: a control group (C) and eleven experimental groups (E_{1-11}) – each comprising 30 birds, all of which were wing tagged. During the first three weeks of the experiment birds were fed a starter diet, followed by a further three weeks on a finisher diet (total of 42 days). Throughout the duration of the experiment, chicks in group C received no rapeseed meal and 1,0% in starter and 0,8% Ca in finisher diets; chicks in group E_{1-11} received a feed mixture containing combination of 10, 20 and 30% rapeseed

Table 1. Distribution of the chicks in the experimental groups according to the different percentage of inclusion of rape seeds (RM) and calcium (Ca) in the diet.

	Groups of chicks											
	C	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_8	E_9	E_{10}	E_{11}
RM	0	0	0	10	10	10	20	20	20	30	30	30
Ca	0.8	1.0	1.2	0.8	1.0	1.2	0.8	1.0	1.2	0.8	1.0	1.2

meal and 1.0, 1.2 and 1.5% in starter and 0.8, 1.0 and 1.2% Ca in finisher diets. Distribution of the chicks in the experimental groups according to RM and Ca is showed in table 1.

Content of total and individual glucosinolates in rapeseed grain before extraction were determined by the method of high-performance liquid chromatography (ISO 9167-1-1992). The total concentration of glucosinolates in Silvia rapeseed meal was 21.78 mmol/kg. Table 2 presents the chemical

Table 2. Performance of the calibration equation used to predict a validation data set.

Groups	TGC mmol/kg	Moisture	Ash	Crude protein	Crude fat	Crude fiber	NFE[1]	Ca	ME[2] MJ/kg
				Starter diets					
C	0.0	12.08	5.44	22.66	4.29	3.66	51.87	1.06	12.23
E_1	0.0	12.04	5.93	22.15	5.02	3.92	50.94	1.25	12.24
E_2	0.0	11.36	6.34	22.36	5.79	3.90	50.25	1.60	12.20
E_3	2.2	11.83	5.17	21.05	5.82	4.33	51.8	1.15	12.26
E_4	2.2	11.36	5.80	22.86	6.40	5.38	48.20	1.23	12.23
E_5	2.2	11.31	6.37	21.35	6.80	5.50	48.67	1.57	12.21
E_6	4.4	11.19	5.48	22.15	6.80	4.31	50.07	1.20	12.11
E_7	4.4	11.88	5.68	22.46	6.32	4.51	48.65	1.25	12.05
E_8	4.4	11.73	6.34	22.66	6.59	4.28	48.40	1.50	11.92
E_9	6.5	10.81	5.42	22.66	8.12	7.59	45.4	1.13	12.06
E_{10}	6.5	10.37	5.49	22.51	8.48	7.40	45.75	1.23	11.99
E_{11}	6.5	10.37	6.76	22.66	8.07	7.26	44.88	1.53	11.87
				Finisher diets					
C	0.0	12.07	5.05	19.44	4.85	4.33	54.26	0.93	12.12
E_1	0.0	11.71	5.06	18.93	5.29	4.60	54.41	1.05	12.05
E_2	0.0	11.75	5.30	18.43	5.52	4.39	54.61	1.28	12.01
E_3	2.2	11.39	5.16	19.93	6.15	5.84	51.53	0.98	12.12
E_4	2.2	11.32	5.48	19.44	6.65	5.68	51.43	1.15	12.08
E_5	2.2	10.62	6.10	19.33	6.87	4.86	52.22	1.25	12.00
E_6	4.4	11.06	5.33	19.94	6.28	5.72	51.67	1.00	12.05
E_7	4.4	11.23	5.37	18.73	6.21	5.61	52.85	1.12	11.98
E_8	4.4	11.26	5.73	18.83	6.96	5.42	51.80	1.33	11.93
E_9	6.5	10.89	5.06	18.98	7.96	4.79	52.32	1.03	12.05
E_{10}	6.5	10.91	5.62	18.73	6.93	4.82	52.99	1.17	11.96
E_{11}	6.5	11.15	6.21	19.03	7.63	4.91	51.07	1.32	11.87

[1] *NFE = nitrogen free-extract.*

[2] *Calculated data from Allen (1993).*

Table 3. Chicks body weight (BW) and daily gain (DG) and at different days of the experiment.

		% of Rapeseed meal (RM)				% of Calcium (Ca)			Significance		
		0	10	20	30	0.8	1.0	1.2	RM	Ca	RMMxCa
BW	g										
d 21	"	613a	631a	612a	577b	640a	620a	564b	*	*	n.s
d 42	"	1790a	1767a	1670b	1630b	1850a	1705b	1588c	*	*	n.s
DG	g/d										
0-21d	"	26.9a	27.8a	26.9a	25.2b	28.2a	27.3a	24.6b	*	*	n.s.
22-42 d	"	55.9a	54.2a	50.4b	50.1b	57.6a	51.7b	48.7c	*	*	n.s.
0-42	"	41.42a	40.99a	38.63b	37.66b	42.88a	39.47b	36.67c	*	*	n.s.

** P<0.05; n.s.- non significant.*
Means with different letters are significantly different

analyses of the starter and finisher diets with total glucosinolate content and with calculated ME.

Chicks were kept in accordance with the recommended Ross technology. Birds were weighed on days 1, 21 and 42, always at the same time of the day and in identical group sequence. The effects of RM and Ca on productive performance were analysed by least-square procedures using General linear Models within PROC GLM of SAS (1999). RM, Ca and their interaction were included in the model as main effects. Effects were considered significant if P<0.05.

Results and conclusions

Mortality was detected only in the starter period and was 0,81% (two chicks E_3, one chick E_{10}). No significant interactions between RM and Ca were detected in the study, so only least square means for RM and Ca are presented in the Table 3.

It was found that chicks in groups fed 0, 10 and 20% of RM realised significantly (P<0.05) higher body weights than those in groups fed 30% of RM at the end of first 21 days of experiment. In the same time average body weights of groups with 1.0 and 1.2% of calcium in diets were significantly (P<0.05) higher than those in groups with 1.5% of calcium in diets. At the end of experiment significantly (P<0.05) higher body weights was found in groups fed 0 and 10% of RM. Our results are consistent with a previous observation by Khan *et al.*, (1996). On the contrary, Richter *et al.* (1996) found lower gains in chicks when inclusion rate of RM in the diet was 5%. Based on these results, we can conclude than 10% (2.2 mmol/kg glucosinolates) of RM in chick's diet didn't have a negative effect on the production traits. More than 0.8% of Ca in the chick's finisher diet caused lower live weight.

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FREE COMMUNICATION

Assessing genetic variability in two ancient chicken breeds of Padova area

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ABSTRACT

Genetic diversity in two ancient indigenous chicken breeds of the Veneto region was assessed using Amplified Fragment Length Polymorphism (AFLP) markers. A total of 63 individuals were analysed using three selected AFLP primer combinations that produced 66 clear polymorphisms. The breeds analyzed were the Padovana and the Polverara (two ancient breeds) and a reference broiler line. The expected heterozygosity (Het) did not differ significantly among breeds. The variability at AFLP loci was largely maintained across breeds, as indicated by the coefficient of genetic differentiation (Gst) value. The lowest genetic distance is found between the Padovana and Polverara breeds suggesting that they could be genetically close.

Key words: AFLP, Indigenous breed, Chicken, Genetic diversity

Introduction

The Padovana and Polverara chicken breeds are phenotypically very similar and their origins are very old; it is thought that the Polverera breed developed by a cross between the Padovana and other local Veneto chicken breeds. It is historically documented that the Padovana breed was introduced to Italy from Poland in 1300 by a Padova noble, Giovanni D'ondi dell'Orologio. The Padovana breed was described for the first time in the Ornitologiae book of Ulisse Aldrovandi (1600) but until 1899 it was still confused with the Polverara breed. Trevisani (1900) and Pascal (1905) were the first authors to describe separately the Padovana and Polverara breeds. The peculiarity of these breeds is the absence of crest that is substitute by a tuft of feathers. The Padovana breed tuft is more pronounced than the Polverara one because the first is caused by a skull hernia. The Padovana breed produces a meat of particular quality (De Marchi *et al.*, 2005), like the Polverara breed, providing a typical product interesting in some local market. Moreover these breeds can be very important biodiversity sources. Indeed biodiversity is essential for the survival of species and populations, and it is assuming greater importance in modern animal science because of an expanding global emphasis on only a few highly selected breeds (Notter, 1999). The aim of this study was to investigate the genetic diversity in the Padovana and Polverara breeds using Amplified Fragment Length Polymorphism (AFLP). AFLP is a multilocus marker technique and employed in several studies to investigate genetic variability at the molecular level also in chicken breeds (Plastow *et al.*, 2003).

Material and methods

Sixty three blood samples were collected from

Table 1. Number of AFLP polymorphisms observed per primer combination within and across chicken breeds.

	Padovana (n = 22)	Polverara (n = 26)	Broiler (n = 15)	Across (n = 63)
E32/T35	13	13	10	20
E45/T32	18	17	11	25
E45/T33	12	15	13	21
Total	43	45	34	66
Mean ± SD	14.3 ± 3.2	15 ± 2	11.3 ±1.5	22 ± 2.6

unrelated male chickens, 15 from a private broiler breeding and 48 belonging to two indigenous chicken breeds: Padovana (22) and Polverara (26) from Co.Va. (De Marchi *et al.*, 2005) project nuclei. The reference breed is a commercial line selected for meat production. Genomic DNA was extracted from whole blood through cells lyses and subsequently precipitated with ammonium acetate. AFLP analysis was carried out following the protocol described in Barcaccia *et al.*, (1999) modified for the use of TaqI endonucleases. TaqI is recommended in mammals (Ajmone-Marsan *et al.*, 1997) and also in poultry (Mock *et al.*, 2002). Sixty three samples were individually assayed with three primer combinations that were tested for the analysis of poultry species and breeds (Ajmone-Marsan *et al.*, 1997). AFLP markers were visually evaluated and scored as dominant markers. To estimate the information content of AFLP markers the following indices were calculated: the number of polymorphisms identified per primer combination within and across breeds, an assay efficiency index (Ai) of the information carried by AFLP markers per analysis (corresponding to a primer combination) as described by Ajmone *et al.*, (1997), average expected heterozigosity (Het) within breeds calculated assuming the population at Hardy-Weinberg equilibrium. Indexes of total (Ht) and within population (Hs) gene diversity were calculated according to Nei (1973), the Gst index (Nei, 1973) was also defined. The following genetic distances between breeds were calculated: standard genetic distance (Nei, 1972), Chord distance (Cavalli-Sforza and Edwards, 1967) and Reynolds distance (1983). The computations were performed by the use of appropriated software (Popgene, Dispan, Phylip).

Results and conclusions

The three primer combinations assayed revealed 66 clear AFLP polymorphisms, with an average of 22±2.6 markers per primer pair and ranging from 21 to 25 (Table 1). The total number of polymorphisms observed within breed ranged from 34 (broiler) to 43 (Padovana).

Only one breed specific marker was detected in the broiler line. Across breeds, the Ai was 32.4, indicating that an average of 32.4 effective alleles (mean: 1.47 alleles per locus) were identified per primer pair (Table 2).

The across breed Ai value of these chicken breeds was lower than that calculated by Ajmone-Marsan (1997, 2001) for other local species. The total and within population gene diversity were

Table 2. Mean and standard error (SE) of expected heterozygosity (Het), average number of effective alleles per locus (ne) and assay efficiency index (Ai) calculated within and across chicken breeds.

Breed	Het (n = 66) (mean ± SE)	Ne (mean ± SE)	Ai
Padovana	0.20 ± 0.02	1.32 ± 0.33	28.9
Polverara	0.21 ± 0.02	1.34 ± 0.34	29.5
Boiler	0.17 ± 0.02	1.27 ± 0.36	26,8
Across breeds	-	1.47 ± 0.31	32.4

Table 3. The Cavalli Sforza and *Reynolds* genetic distances and Nei standard genetic distance.

	Polverara	Padovana
Padovana	0.471 - *0.372* – 0.157	
Broiler	0.735 - *0.498* – 0.246	0.649 – *0.476* - 0.213

respectively 0.291 and 0.188, the Gst value showed that the 35.4% of total variation is accounted by the across breed component. The Gst value reported by Ajmone-Marsan (2001) for the Italian goat populations was lower (0.11) than the value reported in this study. The Polverara breed showed the highest Het index (0.21), followed by the Padovana (0.20) and the broiler line (0.17). The average expected heterozigosity of broiler line was not statistically different from heterozigosity value calculated for the other two indigenous breeds. The Padovana and Polverara breeds showed the lowest genetic distance (Table 3), evidencing to be similar breeds.

The use of AFLP molecular markers permitted a preliminary characterisation of the indigenous chicken breeds of Padova area important for their valorisation, and suggested a genetic similarity between the Padovana and Polverara breeds as reported in historical documents. This study indicates that AFLP is a fast and reliable method to analyse the genome and identify genetic polymorphisms. These markers could be useful for fingerprinting and to estimate genetic relations among individuals and breeds, thus to design marker assisted conservation programmes.

This research was funded by Veneto Agricultural Agency. The authors wish to thank Maristella Baruchello, Pelà Antonio & C. for broiler sample blood and the Polverara chicken association.

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FREE COMMUNICATION

Market of poultry meat and consumers' preferences in the Osijek-Baranja County

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ABSTRACT

Poultry meat is the most consumed type of meat in Croatia. Yearly consumption of poultry meat amounts to 19.3 kg *pro capite*. Consumers prefer price-worthy and tasty meat, which is beneficial to their health. This research into the meat market and consumers' preferences was based on a representative sample of 100 examinees, all residing in the region of Slavonia and Baranja. Among the poultry meat, the majority of examinees most often buy chicken meat (86%), and the rest of them prefer turkey meat (14%). Quality (65%) and price (30%) are the most important factors that influence consumers when buying poultry meat. Almost all consumers prefer to buy poultry meat in its main parts, mostly because of reasonable prices. Even 92% of them decide to buy the most valuable carcass parts (breasts, drumsticks and thighs). In the investigated area of the Osijek-Baranja County, poultry meat is consumed once a week by 86% of examinees.

Key words: Market, Poultry Meat, Consumers, Consumption

Introduction

Poultry production in Croatia has a long tradition, dating back to 15th century. Today, poultry is produced intensively on modern farms, and in small agricultural farms, which apply semi-intensive and free-range rearing systems. According to Croatian statistical records, there were about 11 millions of poultry produced in 2004. In the Statistical Yearbook of the Republic of Croatia (2004) there are no data on division of poultry into specific types and categories. However, it is estimated that in a total population of poultry, chickens take up 93%, followed by geese (3%) and then by turkeys and ducks, taking up 2% each. Other poultry, such as pearl hens, quails, pheasants and pigeons are only an irrelevant part in poultry population (Mužic *et al.*, 2001). Consumption of poultry meat in many developed countries, as well as in Croatia is constantly increasing. Chicken meat is considered to be a dietetic product. It is marked by a high content of valuable protein, and low content of fat, whose profile of fatty acids can be successfully modified as desired (Kralik *et al.*, 2002). Over the last years, it has been noticed that consumers prefer meat that is tasty and, above all, good for their health. In 2003, yearly consumption of fresh meat in Croatia was 51.2 kg *pro capite*. The most consumed meat was poultry (19.3 kg), followed by pork (18.1 kg), and beef (8.6 kg). Consumption of other types of meat is not relevant. In order to obtain an insight into consumers' preferences with respect to poultry meat, a poll was taken on a representative sample.

Material and methods

Official statistical records, as well as previously published papers on related topics were used to ana-

Table 1. Data on poultry produced in Croatia and slaughtered in abattoirs.

Year	Total '000 heads	Poultry slaughtered in abattoirs '000	Net weight, tons
1995	12024	18504	30705
2000	11256	20470	34398
2004	11185	23375*	38944*

Source: Statistical Yearbook (2004) **Data of 2003*

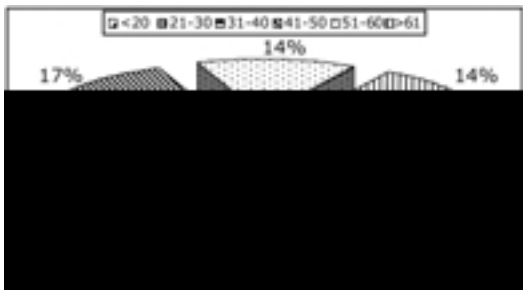
lyze number of poultry, production and market of poultry meat. In order to carry out this research, a poll was taken among residents of the Osijek-Baranja County on their poultry meat consumption preferences. A total of 100 people were involved in the poll, which was based on a questionnaire. All people taking the poll were introduced with the questionnaire content and procedure. A total of 100 people were involved in the poll, out of whom 45% were men and 55% were women. The poll was based on filling out of questionnaires. They answered 11 questions, all of which referred to purchase and consumption of poultry meat, as well as to their preferences regarding a specific type of poultry meat. More than two-thirds of all examinees belonged to the most active age group, between 20 and 50 years of age. Age structure of examinees was presented in Figure 1.

Educational profile of examinees was the following: 4% were with a primary education or less than a primary education, 66% had a high school education, and 30% of examinees had an obtained university or college diploma. Results obtained in the poll were statistically analyzed in MS Office - Excel 2003.

Results and conclusions

According to statistical records on poultry 1995-2004, as presented in Table 1, there is a stagnation noticed in that period. Although poultry is characterized by its fast reproduction, there is still a need to build new or to modernize existing production capacities and technologies in order to increase the poultry production. During 1995-2003, number of slaughtered poultry was increased for 26.3%, the net weight of which was also increased for 26.8%. Considering the fact that 62% of poultry is produced on family-owned farms, it is estimated that overall poultry meat production in Croatia is considerably higher than officially registered by abattoirs.

Figure 1. Age structure of examinees.



Furthermore, Croatia imports live poultry main flocks, and exports broiler meat. Production and consumption balance for poultry meat was as follows: 76746 tons produced, 1536 tons imported and 2466 tons exported (Kolega and Božić, 2001). On Croatian retail market, there are available ready-to-cook and ready-to-grill main chicken parts, and also separately packaged parts (halves, quarters, breasts, drumsticks and thighs, either separated or joint, and wings and backs). Along with all requirements related to quality standards, veterinary and hygienic criteria and nutritional values, special attention is also paid to commercial, technological and culinary properties of meat (Kralik and Petričević, 1994). Results of the poll clearly show that consumers demand quality poultry meat, mostly because they are aware of chronic diseases induced by improper diets (atherosclerosis, diabetes, hypertension, obesity, etc.). Moreover, they are exposed to information on the risks of irregular nutrition. Such constant exposure to information on nutritional properties of poultry meat raised awareness in the community on the importance of consumption of poultry meat and resulted in broad acceptance of poultry meat as a dietetic product of high biological value. When

Figure 2. Consumers' preferences with respect to type of poultry meat.

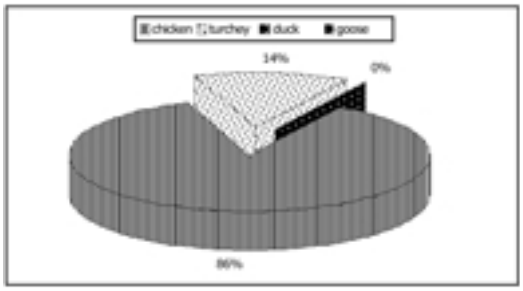


Figure 3. Factors that influence consumers when buying poultry meat.

asked which type of meat they buy mostly among poultry meat, even 86% of consumers selected chicken meat, and only 14% chose turkey meat. None of the examinees buy duck or goose meat (Figure 2). This fact is in accordance with data on relatively small number of produced geese and ducks in Croatia. When buying poultry meat, 66% of consumers pay attention to whether meat was controlled by a veterinary inspection, while 24% find this information irrelevant. If there is a possibility to choose between meat of domestic or foreign production, 77% of examinees will opt for domestic brand products, 20% will decide to buy imported meat, and the rest of 3% will not pay attention to the origin of meat they buy

On the question referring to factors that influence consumers' choice when buying meat, the following answers were obtained: quality does matter to 65% of examinees, 30% decide on the basis of low prices and only 5% select meat because of attractive packaging (Figure 3).

A notion of quality is associated with carcass appearance, commercial brand and product declaration. Free-range broilers are a better marketable product than broilers produced conventionally. In retail supply, almost 92% of consumers buy the most valuable parts, i.e. first class meat, such as breasts, drumsticks and thighs. This is to be justified by reasonable prices of such meat, as well as by consumers' personal preferences. Nutritional ingredients in breasts differ from those in drumsticks and thighs (Giordani *et al.*, 1993). Breasts contain more proteins, K, Mg, SFA and omega-3 FA than meat of drumsticks and thighs. However, dark meat is richer in fat, contains more Na, Zn and Fe, and more omega-6 FA. In comparison with dark meat, the ratio of n-6/n-3 is more favorable in white meat (Leskanich and Noble, 1997, Kralik *et al.*, 2001). Regarding frequency of poultry meat consumption, 7% of examinees consume poultry meat every day, 86% once a week, 7% once a month. Based on the obtained research results, it can be concluded that consumers in Croatia prefer broiler meat, paying attention to quality, commercial brand and veterinary control when buying that meat.

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STATISTICAL YEARBOOK OF THE REPUBLIC OF CROATIA, 2004.

FREE COMMUNICATION

The dietary inclusion of Portulaca oleracea to the diet of laying hens increases the n-3 fatty acids content and reduces the cholesterol content in the egg yolk

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ABSTRACT

The effect of n-3 polyunsaturated fatty acids (PUFA)-enriched diet on yolk fatty acid profile and cholesterol content was evaluated. Dried *Poutulaca oleracea* (purslane: PO diet) was added to a commercial diet (C diet) at 20% of inclusion level. The effect of the supplemented diet was compared to that of C diet. Twenty-six laying hens were fed *ad libitum* for 21 days with the 2 diets, supplemented also with 300 mg α-tocopherol acetate/kg. Eggs were collected and then the fatty acids (FA) profile and the cholesterol content were analysed. The PO diet significantly reduced the saturated FA content ($P<0.05$) and increased that of the polyunsaturated FA (PUFA): 18:2 n-6 ($P<0.001$), 18:3 n-3 ($P<0.001$) and 22:6 n-3 (DHA; $P<0.01$). Both n-6 and n-3 PUFA significantly increased with the PO diet and the n-6/n-3 ratio was improved (10.4 *vs* 11.3; $P<0.05$).

Key words: Laying hens, *Portulaca oleracea*, N-3 fatty acids, Yolk

Introduction

Portulaca oleracea (PO) is an herbaceous weed that contains many biologically active compounds and is a source of many nutrients. PO is one of the richest green plant sources of omega-3 fatty acids, such as alpha-linolenic acid (18:3 n-3), but contains also high levels of linoleic acid (18:2 n-6), both essential for normal human growth, health promotion, and disease prevention. PO leaves have also high contents of antioxidants, such as alpha-tocopherol, ascorbic acid and glutathione (Simopoulos *et al.*, 1992). The high levels of protein in PO compete with those of other commercially important vegetable crops and it has been measured from 20 to 27% according to the planting date (Ezekwe *et al.*, 1999). Fed to hens, PO could be able to increase the omega-3 fatty acids and to reduce the cholesterol content of the eggs yolk. To date, any study involved the effect of the PO supplement fed to hens on their egg composition.

Material and methods

With the aim to increase the levels of n-3 FA and to reduce the cholesterol content in eggs, 2 diets containing different levels of polyunsaturated fatty acids n-3 (PUFA n-3) were tested. The control diet was a commercial diet (C) while the other diet was the C diet supplemented with 20% of dried *Portulaca oleracea* (purslane: PO). Twenty-six laying hens from Warren strain of 24 weeks of age were fed *ad libitum* for 21 days the 2 diets, enriched also with 300 mg a-tocopherol acetate/kg.

Table 1. Chemical composition of the experimental diets (as fed).

		Diets	
		C	PO
Inclusion level	%	--	20.0
Moisture	"	9.0	8.3
Crude Protein (calculated)	"	17.5	18.8
Ether Extract	"	5.6	3.4
Ash	"	12.8	16.6
Crude fibre	"	2.9	5.2
N-free Extracts	"	51.2	46.8
NDF	"	14.0	16.2
ADF	"	3.5	6.5
ADL	"	0.34	1.89
Calculated ME [(1)]	MJ/kg	12.87	11.04

[(1)]*Sibbald,1980*

Fifty-four eggs from C and 24 from PO dietary groups were collected and then analysed.

Diets were analysed (Table 1) to determine moisture, ether extract, ash, crude fibre, N-free extracts and fibre fractions, while protein was calculated by difference according to the standards of the A.O.A.C. (1984). The metabolizable energy (ME) was calculated with the equation of Sibbald (1984). The fatty acids (FA) profile of diets and egg yolks were determined using a gas-chromatography, after Folch extraction, according to A.O.A.C. methods (1984). The cholesterol content of yolks was also determined (Casiraghi *et al.*, 1994). The data were submitted to ANOVA (SAS, 1990) adopting a linear model which considered the diet effect.

Results and conclusions

Poutulaca oleracea at 20% of inclusion level modified the FA profile of the PO diet, if compared to the control diet (Table 2). In particular, the dietary PUFA content increased from 32.2 to 46.5% total FA, due to the enhancement of C18:2 n-6 (41.7 *vs* 30.4% total FA) and C18:3 n-3 (4.77 *vs* 1.87% total FA). Consequently, the saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) decreased. This PO inclusion level lead to an important dietary improvement of the n-3 FA

Table 2. Fatty acid profile of experimental diets (% total FA).

	Diets	
	C	PO
Saturated Fatty Acids (SFA)	33.4	28.2
C12:0	0.00	0.13
C14:0	1.57	1.26
C16:0	21.8	21.1
C18:0	10.1	5.7
Monounsaturated Fatty Acids (MUFA)	34.4	25.2
C16:1	1.74	0.91
C18:1 n-9	32.6	24.3
Polyunsaturated Fatty Acids (PUFA)	32.2	46.5
C18:2 n-6	30.4	41.7
C18:3 n-3	1.87	4.77
Total UFA	66.6	71.7
UFA/SFA	1.99	2.54
n-6	30.4	41.7
n-3	1.87	4.77
n-6/n-3	16.2	8.8

Table 3. Fatty acid composition and cholesterol content of yolk.

Diet	C	PO	P-value[a]	RMSE
Eggs, n	54	24		
SFA	44.1	43.0	*	2.0
C14:0	0.28	0.25	**	0.04
C16:0	29.3	29.8	ns	1.3
C18:0	14.5	14.4	ns	1.0
MUFA	38.1	37.6	ns	2.2
C16:1	2.09	2.00	ns	0.37
C18:1	36.0	35.0	*	1.5
PUFA	17.8	19.4	***	1.3
C18:2 n-6	12.0	13.2	***	0.9
C18:3 n-3	0.18	0.30	***	0.11
C20:4 n-6	4.34	4.40	ns	0.53
C22:6 n-3	1.28	1.48	**	0.25
UFA	55.9	57.0	*	2.0
UFA/SFA	1.27	1.34	*	0.12
n-6	16.4	17.6	***	1.2
n-3	1.46	1.80	***	0.24
n-6/n-3	11.3	10.4	*	1.8
PCL/PCE[(1)]	1.25	1.28	ns	0.08
Cholesterol[(2)], mg/egg	301	266	†	33

[a] *†: P<0.10; *: P<0.05; **: P<0.01; ***: P<0.001;*

[(1)] *Plasma Cholesterol Lowering FA/ Plasma Cholesterol Elevating FA = (PUFA + 1/2 MUFA) / (C12:0 + C14:0 + C16:0);*

[(2)] *analysed on 8 C and 6 PO yolks*

and to a quite halved n-6 to n-3 ratio.

The differences in the FA profile of the C and PO diets were maintained in the FA profile of the eggs of hens fed the 2 experimental diets. In particular, the yolks of hens receiving the PO diet showed a significant reduction in the saturated FA content (43.0 *vs* 44.1% total FA; P<0.05) and an increase in the PUFA content (19.4 *vs* 17.8% total FA; P<0.001). Within the PUFA, the increase involved the C18:2 n-6 (13.2 *vs* 12.0% total FA; P<0.001), the C18:3 n-3 (0.30 *vs* 0.18% total FA; P<0.001) and the C22:6 n-3 (DHA: 1.48 *vs* 1.28% total FA; P<0.01). Both n-6 and n-3 PUFA significantly increased with the PO diet, but the ratio n-6/n-3 was healthily improved (10.4 *vs* 11.3; P<0.05).

As epidemiological studies indicates that n-3 FA exert protective effects against some common cancers, especially cancers of the breast, colon and prostate, these findings should serve as a strong incentive for the use of PO in the hens feeding. Portulaca oleracea should be considered as an item of agriculture and commerce and, on the basis of the results emerged in this study, it could be used for the poultry feeding.

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FREE COMMUNICATION

Organic farming production. Effect of age on the productive yield and egg quality of hens of two commercial hybrid lines and two local breeds

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ABSTRACT

Hens of 2 hybrid lines (Hy Line Brown - HLB, Hy Line White - HLW) and 2 local breeds (Robusta maculata – RM and Ermellinata of Rovigo – ER) were reared following the organic production procedure and their egg yield and quality were studied at two different ages (30 and 42 weeks). The experimental period was between 26 and 42 weeks of age, starting in summer and lasting until autumn. Age significantly ($P<0.01$) affected the hen-day egg production and the daily egg mass of the HLB (84 *vs*. 94% and 44.5 *vs*. 59.4 g), of the HLW (81 *vs*. 89% and 42.4 *vs*. 54.7 g) and of the RM hens (27 *vs*. 63% and 12.9 *vs*. 36.7 g), which was the least precocious. Age did not affect the daily egg production of the ER hens (which was maintained around 58%), although it was observed a slight ($P<0.05$) increase of the daily egg mass (28.6 *vs*. 32.1 g). Age increased ($P<0.01$) the egg weight, yolk colour, yolk percentage and yolk:albumen ratio and decreased the albumen percentage in all groups.

Key words: Organic farming, Laying hen, Genotype, Egg quality

Introduction

In the organic farming production regulation (OJEC, 1999) the use of local breeds is preferred as respect to the use of other genotypes because they are supposed to be more adaptable and rustic under less controlled conditions of rearing. For intensively reared commercial hybrid lines (Bell, 2002) the effect of age and environment on the quality of eggs is well known.

In this trial the effect of age on the productive performance and on the quality of eggs of hens belonging to two hybrids and two local breeds kept under organic farming circumstances was evaluated in the first phase of oviposition.

Material and methods

Laying hens of four genetic types (70 birds per type): 2 commercial hybrid lines, brown egg line (Hy-Line Brown- HLB) and white egg line (Hy-Line White - HLW) and two Italian (Veneto) dual-purpose breeds, Robusta maculata (brown egg – RM) and Ermellinata of Rovigo (light-brown egg - ER) were reared under the same circumstances. The rearing conditions followed the organic production regulation (OJEC, 1999). The trial started in summer when the animals were 26 weeks old and lasted in autumn at 42 weeks of age. The environmental conditions changed widely throughout the experimental period: the temperature and the relative humidity ranged from about 30 to 5°C and from 70 to 75%, respectively. The photoperiod was maintained constant (16 h of light and 8 h of dark) by means of artificial light according to the season. The hens received *ad libitum* the same diet through the whole experimental period (crude protein=18.70%; metabolizable energy=11.82 MJ/kg; calcium=3.80%;

Table 1. Effect of age on productive yield of the laying hens.

	HLB		HLW		RM		ER	
	30 wk	42 wk	30 wk	42 wk	30 wk	42 wk	30 wk	42 wk
Hen-day egg yield, %	84.2^{A}	94.1^{B}	80.7^{A}	88.7^{B}	27.1^{A}	63.0^{B}	59.0	56.8
Root MSE	9.27	9.14	10.90	9.59				
Egg mass, g/d	44.5^{A}	59.4^{B}	42.4^{A}	54.7^{B}	12.9^{A}	36.7^{B}	28.6^{a}	32.1^{b}
Root MSE	5.13	5.09	5.93	5.36				
Feed:egg, g/g	1.89^{A}	2.48^{B}	2.17	2.10	9.26^{B}	3.61^{A}	3.13^{A}	4.30^{B}
Root MSE	0.3584		0.2259		3.9183		0.7753	

$^{a, b}$: P<0.05; $^{A, B}$: P<0.01; HLB=Hy Line Brown, HLW=Hy Line White, RM=Robusta maculata, ER= Ermellinata of Rovigo; 30 and 42 wk=30 and 42 weeks of age; degrees of freedom=54

phosphorus=0.70%). The hen-day egg production (number of eggs/number of live hens x 100), the daily egg mass and the feed:gain ratio were recorded during the four weeks preceding the 30th and the 42nd week of age. Samples of about 30 eggs per genotype, collected at 30 and 42 weeks of age, were analysed to evaluate the weights of egg, yolk, albumen and eggshell, the yolk colour (by means of a colour Roche scale) and the eggshell thickness (by means of a digital micrometer).

Data were subjected to analysis of variance (SAS, 1996).

Results and conclusions

Age significantly (P<0.01) increased the hen-day egg production (Table 1) in both the two hybrid lines and in RM, where the daily egg yield doubled passing from 30 to 42 weeks of age. The daily egg yield of ER was unaffected by age.

Age had significant effects on the daily egg mass in all the genetic lines: HLB and HLW presented a similar increase (P<0.01), the RM hens showed a final egg mass about 3 times higher (P<0.01) than that observed at 30 weeks of age, whereas the increase of the ER daily egg mass was less marked (P<0.05). These data indicated that, under the rearing circumstances adopted, the hybrid lines reached the peak of the production curve later than the age normally reported by Hy Line Ltd. RM hens started laying later with respect to the other groups and the number and the weight of the eggs increased markedly with age. The ER hens demonstrated a rather constant production from 30 to 42 weeks of age.

Age had also significant effects on the measured feed:egg ratio which increased in HLB and in ER and decreased in RM. No effect of age on this parameter was observed for the HLW group of hens. The increase of the feed:egg ratio according to the age and the season, in the HLB and ER groups could be due to an higher energy and nutrient demand for thermoregulation and body growth. At 42 weeks the RM hens showed, as respect to the earlier age, a more favourable feed:egg ratio, probably due to a decreasing incidence of energy and nutrients used for growth as respect to that used for egg production. These results are in agreement with the observation that during the trial the local dual-purpose breeds presented higher body gain with respect to the hybrid lines (Rizzi *et al.*, 2004). The RM hens probably present a higher and more precocious muscle growth and start laying eggs later, and the ER hens start egg production earlier but present a more gradual development of body weight in comparison to the RM hens.

In table 2 some egg quality parameters are reported. Age significantly (P<0.01) influenced the egg weight in all groups. The effect of age on eggshell quality was limited: the thickness did not change with the exception of the HLB eggs which showed lower (P<0.05) values at 42 weeks.

The incidence of the eggshell on the total weight of the egg decreased (P<0.05) only in the HLB and ER groups. The yolk colour increased (P<0.01) with the age of the animals in all groups.

Age significantly (P<0.01) increased the proportion of yolk and decreased (P<0.01) the incidence of albumen, particularly in the local breeds.

The yolk:albumen ratio was always increased by age (P<0.01), but the ER and RM eggs showed the highest values.

Age may affect the quality of the egg: the yolk colour is affected not only by the intake of carotenoid pigments but also by the body synthesis of lipids. In agreement

Table 2. Effect of age on various egg quality parameters.

	Egg weight g	Eggshell thickness µm	Yolk colour Roche	Yolk %	Albumen %	Eggshell %	Yolk:Albumen ratio
HLB							
30 *wk*	56.9 ^{A}	376^{b}	7.30^{A}	23.36^{A}	65.22^{b}	11.42^{B}	0.36^{A}
42 *wk*	66.11^{B}	364^{a}	9.70^{B}	25.23^{B}	64.30^{a}	10.47^{A}	0.39^{B}
Root MSE	3.88	19	0.5960	1.2918	1.5389	0.8708	0.0286
HLW							
30 *wk*	55.5^{A}	342	7.20^{A}	24.47^{A}	65.22^{B}	10.31	0.38^{A}
42 *wk*	63.9^{B}	347	9.64^{B}	27.13^{B}	62.55^{A}	10.32	0.44^{B}
Root MSE	3.89	22	0.6973	1.5029	1.5356	0.5674	0.0335
RM							
30 *wk*	52.5^{A}	373	7.35^{A}	26.33^{A}	62.85^{B}	10.82	0.42^{A}
42 *wk*	60.3^{B}	383	10.21^{B}	29.77^{B}	58.93A	11.30	0.51^{B}
Root MSE	2.93	28	0.8068	1.4415	1.5114	0.8838	0.0366
ER							
30 *wk*	51.2^{A}	338	7.55^{A}	27.91^{A}	62.00^{B}	10.08^{B}	0.45^{A}
42 *wk*	58.8^{B}	322	10.00^{B}	31.40^{B}	59.10^{A}	9.50^{A}	0.53^{B}
Root MSE	3.69	33	0.7818	1.4888	1.7915	0.7936	0.0396

$^{a, b}$: P<0.05; $^{A, B}$: P<0.01; HLB=Hy Line Brown, HLW=Hy Line White, RM=Robusta maculata, ER= Ermellinata of Rovigo; 30 and 42 wk=30 and 42 weeks of age; degrees of freedom: HLB=58; HLW=56; RM=44; ER=51

with the results of Silversides and Scott (2001), the weight of yolk increased with age more than did the albumen. In our trial the higher increasing of weight were observed for the yolks of the local pure breeds. These results confirm that the genetic improvement of selected lines of hens has involved the weight of the egg and their protein fraction rather than the lipid fraction (Suk and Park, 2001). So the hybrid lines, especially HLB, present a yolk incidence lower than that of local breeds.

The data demonstrate that in the first phase of production, from 30 to 42 weeks of age, and under our experimental conditions, significant change in the egg yield and quality can be expected: the commercial hybrid lines and the RM breed increased the number of eggs produced per day; the daily egg mass increased in all the four genetic stocks. The feed:egg ratio increased in the HLB and ER hens which presented higher body growth than HLW, whereas in the RM hens the ratio decreased because these hens started laying later than those of the other groups. Age increased the egg weight, yolk colour and yolk:albumen ratio in all groups; the eggshell thickness and eggshell percentage decreased in the HLB and ER eggs. In conclusion this work provides some information which can be useful for the organic farmers to improve their strategy of production and marketing.

The authors want to thank Veneto Agricoltura for its financial support.

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FREE COMMUNICATION

The crossbreeding of improved Jezersko-Solčava sheep with Charollais to improve carcass traits

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ABSTRACT

Thirteen improved Jezersko-Solčava lambs (JSR) and 16 crossbreds with Charollais (JSRxCH) were used to evaluate the effect of genotype and sex on carcass traits. Lambs were weaned at around 60 days of age. They were fed with commercial concentrate and hay ad libitum and slaughtered at 30 kg of average live weight at 103 days of age. Crossbred lambs had at the same live weight at slaughter better conformation (9.19 on 15 points scale) than JSR lambs (7.58). Carcasses of crossbred lambs had lower proportion of fat in hind leg and lower proportion of kidney fat. Female lambs had better dressing proportion (46.25%) than male lambs (42.72%). Females had also higher scores for both internal and subcutaneous fatness and higher percentage of kidney fat. They had higher proportion of fat and lower proportion of bone in hind leg.

Key words: Lambs, Genotype, Sex, Carcass traits

Introduction

The present trend in Slovenia is to slaughter lambs at 30 kg of live weight (around 100 days of age). Breeders are using traditional husbandry system. They wean lambs at around 50 to 60 days and then fatten them on cereals or commercial concentrate till they reach 30 kg. The prevalent breeds in Slovenia are autochthonous Jezersko-Solčava breed (JS) and Jezersko-Solčava breed improved with Romanov breed (JSR). JSR breed is known as a very fertile breed (Cividini et al., 2002), but on the other hand its carcass quality is not so well known. A great number of factors affect ovine carcass and meat quality (Sanudo et al., 1998, Alfonso et al., 2001). Carcass quality is also effected by the breeder and his choice of animal genotype (Hopkins et al., 1997, Hawkins et al., 1985), and breeding technology (Santos-Silva et al., 2002, Diaz et al., 2002). One of the fastest ways to improve carcass quality represents crossbreeding, mostly industrial crossbreeding. Therefore industrial crossbreeding with Texel and in 2004 with Charollais breed was introduced in Slovenia. The aim of the following research was to compare carcass traits of improved Jezersko-Solčava lambs and their crossbreds with Charollais breed and to evaluate the effect of sex on these traits.

Material and methods

In the experiment 29 lambs of two genotypes were included: 13 improved Jezersko-Solčava lambs (JSR), 6 males and 7 females, and 16 crossbred lambs between improved Jezersko-Solčava

Table 1. Carcass traits in improved Jezersko-Solčava lambs and their crossbreds with Charollais (LSMeans ±SEE).

Carcass traits		Genotype (G)		Sex (S)		Level of significance	
		JSR (N= 13)	JSRxCH (N = 16)	Male (N = 14)	Female (N = 15)	G	S
Live weight at slaughter	kg	29.79 ± 0.39	30.19 ± 0.35	30.27 ± 0.37	29.71 ± 0.36	NS	NS
Daily weight gain	g	245 ± 10	268 ± 9	272 ± 10	240 ± 10	NS	*
HCW	kg	13.40 ± 0.26	13.28 ± 0.23	12.92 ± 0.25	13.75 ± 0.24	NS	*
DP	%	44.96 ± 0.50	44.00 ± 0.45	42.72 ± 0.48	46.25 ± 0.47	NS	***
EUROP-conform.	score	7.58 ± 0.45	9.19 ± 0.40	7.96 ± 0.44	8.81 ± 0.42	**	NS
EUROP-fatness							
- subcutaneous	score	6.82 ± 0.40	7.06 ± 0.36	6.31 ± 0.39	7.57 ± 0.38	NS	*
- internal	score	6.17 ± 0.39	5.50 ± 0.35	4.87 ± 0.38	6.80 ± 0.36	NS	**

*HCW: hot carcass weight; DP: Dressing proportion; EUROP-fatness: 5_+=15, 5_0=14, 5-=13, 4_+=12, 4_0=11, 4-=10, 3_+=9, 3_0=8, 3-=7, 2_+=6, 2_0=5, 2-=4, 1_+=3, 1_0=2, 1-=1; EUROP-conformation: E_+=15, E_0=14, E-=13, U_+=12, U_0=11, U-=10, R_+=9, R_0=8, R-=7, O_+=6, O_0=5, O-=4, P_+=3, P_0=2, P-=1; *P<0.05; **P<0.01; ***P<0.001; NS: not significant*

breed and Charollais (JSRxCH), 8 males and 8 females. Lambs of both genotypes and sexes were taken from two breeders (2x2x2 factorial design), reared on the farm where they were born, until slaughter. Lambs were reared with their dams on the pasture up to the age around 60 days (about 20 kg live weight), at which they were weaned. From 10 days on ward, the lambs were fed with commercial concentrate (18% crude proteins, 8% crude fibrins) and hay ad libitum until slaughter.

After slaughter, hot carcass weight (HCW) was determined. Kidney with knob channel fat belongs to the carcass. Carcass conformation and fatness classes were subjectively scored according to EUROP classification, with three subclasses in each class (points from 1 to 15).

Carcasses were kept at room temperature for 2 h, and where then chilled at 4°C for 24 hours. After chilling cold carcass weight was measured and further separated into main joints. Right leg was further dissected into muscle, subcutaneous fat and bone. Data were analyzed using the GLM procedure of SAS (1990). Fixed effects of genotype, sex and breeder were included in the model.

Results and conclusions

Mean slaughter weight of JSR lambs was 29.79 kg (108 days old) and the mean weight of crossbred lambs was 30.19 kg (100 days old).

Mean values for all carcass traits are shown in Table 1. Jezersko-Solčava lambs and their crossbreds with Charollais had similar growth rate and were slaughtered at around 30 kg live weight. Contrary to our expectation there were no significant differences in dressing proportion between JSR lambs and their crossbreds with Charollais. Santos Silva et al. (2002), Carson et al. (1999), Hawkins et al. (1985) and Cividini (2004) reported that crossbreds with terminal meat breeds used in their experiment reached better dressing proportion.

Crossbred lambs had higher values for carcass conformation at the same degree of fatness. Male lambs grew faster and had higher daily gain from birth until slaughter. Females had also better dressing proportion and so higher hot carcass weight. Female lambs were fatter than male lambs as indicated by external and internal fat estimation. Higher carcass fatness in females was also found by Vergara & Gallego (1999).

The proportion of fat in hind leg and the proportion of kidney fat differed between crossbred lambs and purebred JSR lambs (Table 2). Lower proportion of fat in hind leg and kidney fat was attained in crossbred lambs. The composition of hind leg showed that females had higher ($p<0.001$)

Table 2. Proportion of kidney fat, proportion of hind leg and its tissue composition of improved Jezersko-Solčava lambs and their crossbreds with Charollais (LSMeans ±SEE).

Carcass traits		Genotype (G)		Sex (S)		Level of significance	
		JSR (N= 13)	JSRxCH (N = 16)	Male (N = 14)	Female (N = 15)	G	S
HCW	kg	13.40 ± 0.26	13.28 ± 0.23	12.92 ± 0.25	13.75 ± 0.24	NS	*
Kidney fat	%	1.74 ± 0.10	1.27 ± 0.09	1.22 ± 0.10	1.79 ± 0.10	**	***
Hind leg	%	30.38 ± 0.48	31.04 ± 0.43	31.00 ± 0.47	30.43 ± 0.45	NS	NS
Hind leg composition							
muscle	%	70.88 ± 0.48	71.80 ± 0.43	71.83 ± 0.46	70.86 ± 0.45	NS	NS
fat	%	10.63 ± 0.36	9.61 ± 0.32	8.95 ± 0.34	11.30 ± 0.33	*	***
bone	%	18.50 ± 0.34	18.58 ± 0.31	19.22 ± 0.33	17.84 ± 0.32	NS	**

*HCW: cold carcass weight *P<0.05; **P<0.01; ***P<0.001; NS: not significant*

fat proportion and lower (p<0.01) bone proportion. Females had also more kidney fat. Similar results were found also by Hawkins et al. (1985).

On the basis of attained results, we can conclude that industrial crossing of JSR ewes with Charollais rams improved carcass quality of lambs slaughtered at 30 kg of live weight. Crossbred lambs had better carcass conformation and lower proportion of fat in hind leg. At the same live weight female lambs exhibited higher dressing proportion and higher fatness.

This work was supported by the Ministry of agriculture, forestry and food and Ministry of science and technology of the Republic of Slovenia.

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FREE COMMUNICATION

Effects of extruded corn on milk yield and composition and blood parameters in lactating dairy cows

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ABSTRACT

According to a 2x2 cross over design, fourteen Holstein dairy cows at 99±55 DIM were fed two diets containing 21.5% DM of either ground corn (GC) or extruded corn (EC). Performance and metabolic profile were detected during the third week of each experimental period. DMI and milk yield were not affected by dietary treatments. Milk fat and protein percentage of EC diet were significantly ($P<0.10$) lower than those of GC diet. Probably the higher rumen degradability of starch from EC thesis modified the synthesis of specific fatty acids leading to a milk fat depression event. Diets did not influence blood parameters, except for lower values of total protein and glucose content in EC diet-fed cows. Results suggested that the dietary inclusion of extruded corn should not be used at the tested level of substitution.

Key words: Corn extrusion, Dairy cows, Milk quality, Plasma parameters

Introduction

High producing dairy cows need high energy concentration in diets. This objective can be achieved by dietary inclusion of large amount of grains, and/or improving their starch availability. Corn is the most important grain for dairy cow feeding since it is cheap and spread, even if its starch degradability is lower than that of other cereals (barley, sorghum). Starch ruminal degradation of grain can be increased by physical treatments (grinding, crushing, rolling) or involving the addition of heat, water and pressure (flaking, extrusion). In general, extrusion processing improves corn starch availability more than other physical treatments (Gaebe *vs*, 1998). It seems that intestinal digestive capacity by amylase is the primary limitation to total absorption of monosaccharides from starch digestion. Thus, dairy cows benefit more from a supply of quickly fermented starch than from an increased supply of ruminal by-pass starch (Huntington, 1997). An increase of readily degradable starch allows the improvement of nutrient production by rumen fermentation. However, the use of high degradable starch on lactating dairy cow diets causes uncertain effects on performance (Theurer *vs*, 1998). The objective of the present study was to assess the effects of a complete substitution of ground corn with extruded one on milk production and composition and metabolic profiles in Holstein dairy cows.

Material and methods

Fourteen lactating Italian Holstein dairy cows at 99±55 DIM were used in a 2x2 cross over design with three weeks periods. Two experimental diets based on corn silage (33.5% DM), hay (12.2% DM) and alfalfa silage (7.6% DM) were formulated: GC using 21,5% of ground corn and EC by using the same percentage of

Table 1. Chemical composition of the experimental diets.

		Experimental diets	
		Extruded Corn	Ground Corn
Dry Matter	%	51.8	52.4
Crude Protein	% DM	15.6	15.5
Ether Extracts	"	4.3	4.0
Ash	"	7.2	7.1
NDF	"	35.2	35.8
ADF	"	21.6	18.1
NSC[1]	"	37.7	37.6

[1] *Non structural carbohydrates*

extruded corn. Diet samples were dried, ground and analyzed for proximate composition (AOAC, 2000); NDF and ADF were evaluated by ANKOM method (Table 1). Cows were fed *ad libitum* and DM intake (DMI) was individually and continuously recorded by *BIOcontrol sistem* A/S (Grimstad Gard, Norway). Cows were milked twice daily and milk yield was recorded at each milking over the last five days of each period. Milk samples from two daily milking were collected on the 16th and 18th day and analyzed for fat, protein, lactose (midinfrared spectroscopy) and milk urea nitrogen (MUN, enzymatic method). At the 17th day, blood samples were collected from jugular vein by venipunctur into heparinized tubes at 9:00 and 14:00 h. Plasma was analyzed for the following parameters: total protein, albumin, plasma urea nitrogen (PUN), glucose and NEFA. Data were analyzed by ANOVA (GLM of SAS) according to the following model: Yijkl = μ + Di + Cj + Pk + eijkl, where Yijkl is the variable, μ is the overall mean, Di is the diet effect, Cj is the cow effect, Pk is the period effect and eijkl is the residual error.

Results and conclusions

Dry matter intake (DMI) was not influenced by diets (Table 2). Many works on dairy cows (Lykos *vs*, 1997; Knowlton *vs*, 1998) reported no difference on DMI in relation to different corn starch digestibility. Reduction in DMI was often observed using diets characterized by a more rapid starch degradability both in dairy cows (Yu *vs*, 1998) and steers (Gaebe *vs*, 1998). Any significant difference was found between treatments in milk yield according to literature (Crocker *vs*, 1998). However, Shabi *vs* (1999) found a decrease of DMI and milk yield using an experimental diet with 20% of extruded corn. Fat corrected milk (FCM) was lower on EC diet (28.5 *vs*. 30.4 kg/d; P<0.05) because of fat percentage reduction (3.91 *vs*. 4.15%; P<0.10). Theurer *vs* (1998), in a wide review, reported a reduction of milk fat content if cows were fed with a higher amount of degradable NSC, even if the phenomenon was always associated with a dietary NDF value lower than that used in this experiment. Since extrusion increases starch rumen degradability, probably EC diet-fed cows reached a lower ruminal fluid pH. As a consequence, a higher production of trans fatty acids (particularly C18:2 trans-10-cis-12) could have inhibited one o more steps of fat synthesis by mammary gland (Baumgard *vs*, 2000). Thus, the observed reduction of milk fat percentage probably is explained by biohydrogenation theory of milk fat depression (MFD). Milk protein percentage of EC diet tended to be lower than that of GC (3.58 *vs*. 3.70%; P<0.10). As confirmed by similar milk urea nitrogen (MUN) values between diets, probably the total substitution of ground corn by extruded one did not alter nitrogen metabolism. This result was unexpected: as provision of high degradable starch increases milk protein percentage, MUN concentration should decrease (Lycos *vs*, 1997). Plasma total protein was significantly lower in EC-fed cows (76.2 *vs*. 79.5 g/l; P<0.05) because of a tendentious reduction of both albumin and globulin concentration. Plasma glucose concentration was higher in GC thesis (3.38 *vs*. 3.61 mmol/l; P<0.01), whereas concentration of NEFA was not affected by dietary treatment. These results are not in agreement with Lykos *vs* (1997) and Khorasani *vs* (1994) who found a NEFA linear decreasing and a stable glucose concentration as a more degradable starch level has been fed. More over, a change on NEFA plasma concentration could occur if dietary starch availability influenced milk fat percent-

Table 2. Dry matter intake, milk production and plasma parameters.

		Experimental diets		*P*	SEM
		Extruded Corn	Ground Corn		
Dry matter intake	kg/d	23.3	24.0	ns	0.4
Milk yield	"	29.4	30.0	ns	1.5
FCM[1]	"	28.7	30.6	*	0.5
Milk fat	%	3.91	4.15	†	0.09
Milk protein	"	3.58	3.70	†	0.04
Milk urea	mg/100 ml	29.6	27.6	ns	4.5
Plasma parameters					
Total protein	g/l	76.2	79.5	*	1.0
Albumin	"	33.8	34.6	ns	0.4
Urea	mmol/l	5.85	5.66	ns	0.19
Glucose	"	3.38	3.61	**	0.03
NEFA	"	0.09	0.07	ns	0.02

[1] *4.0% Fat Corrected Milk; †: P<0.1; *: P<0.05; **: P<0.01*

age. An increase in the proportion of dietary concentrate often results in a higher plasma glucose concentration (Dhiman *vs*, 1991). Since cows were fed diets having the same concentrate level, the significant different plasma glucose concentration observed was probably due specifically to the kind of corn treatment. As expected, plasma urea nitrogen (PUN) and MUN were similar between the two theses since PUN is the main source of MUN.

Summarizing, experimental data suggested that a complete (as 21.1% of DM) substitution of ground corn seed with extruded one did not affect dry matter intake and milk production. As a consequence of extrusion, the increase of degradability rate of starch induced a reduction of milk fat percentage and afterwards a FCM decrease. Probably a change in ruminal fermentation occurred, modifying the synthesis of chemical constituent of milk. In feeding lactating dairy cows, the dietary inclusion of extruded corn should not be used at the experimental level tested in the present trial.

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FREE COMMUNICATION

Genetic aspects of milk electrical conductivity in Italian Brown cattle

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ABSTRACT

Electrical conductivity (EC) of milk is defined as an indicator of the udder health. The EC is a low cost and easy recordable information in dairy herds with automatic milking systems. The heritability of EC showed to be higher than somatic cell count and for this reason might be an useful trait for indirect selection for mastitis resistance. The heritability for EC in Italian Brown cattle was equal to 0.23. Therefore, EC could be useful not only for dairy cows management but also for selection of dairy cows. The high correlation between breeding values for SCC and EC is promising in order for improving mastitis resistance and functional ability of dairy cows.

Key words: Dairy cattle, Electrical conductivity, Mastitis, Genetic parameters

Introduction

Electrical conductivity (EC) of milk could be considered a mastitis indicator (Norberg et al., 2004). The EC value depends on the concentration of anions and cations in milk. The concentration of Na^+ and Cl^- increases in milk produced under mastitis disease; therefore, milk from mastitis cows typically shows higher EC when compared to milk from health cows (Kitchen, 1981) and the control of milk EC has been proposed as a tool for monitoring udder health in dairy cows (Norberg *et al.*, 2004). In modern dairy herds automatic systems are spreading for monitoring several traits related to yield, fertility and health status of dairy cows (De Mol *et al.*, 1997). Namely, there are currently some milking automatic systems that allow to collect individual total milking time, milk yield and milk EC, at every milking. Therefore, EC could became a low cost and easy recordable information for several dairy herds; as EC showed in Holstein cows a moderate to high heritability and positive correlation with mastitis (Norberg *et al.*, 2004), it could be useful not only for dairy cows management, but also for selection of dairy cows.

Given these premises, this study aimed to analyse the sources of variation and to investigate genetic parameters of EC collected through automatic milking systems on Italian Brown cows reared in four herds of Trento Province.

Material and methods

This study involved four Italian Brown Swiss dairy herds of Trento Province with a size ranging between 25 and 123 milking cows. Individual total milking time (TMT), milk yield (MY) and the highest EC value per cow were collected at each milking between May 2002 and July 2004 on all milk-

Table 1. Descriptive statistics for total milking time (TMT), logarithm of TMT, electrical conductivity (EC), logarithm of EC and milk yield (MY).

Informatics data	Average	S.D.	Min	Max
TMT	6.6	2.0	2.0	15.0
Ln(TMT)	1.84	0.30	0.69	2.71
EC	494	63	200	800
Ln(EC)	6.20	0.12	5.30	6.69
MY	12.8	3.6	3.0	25.0

ings. Data were collected using the Dairy Plan Software (Westfalia®) and were edited using Microsoft Visual Fox Pro procedure. Only milking session with at least ten records and data relevant to cows with at least 15 records, with days in milk ranging between 5 and 400 days, with TMT values ranging between 2 and 15 minutes, with MY values ranging between 3 and 25 kg, with EC values ranging between 2 and 8 ms/cm and with a milking interval from previous milking of at least 7 hours were retained for analysis. After editing 115,799 milking records involving 429 lactations and 233 cows were available. TMT [Ln(TMT)] and EC [Ln(EC)] were log transformed to normalize the distribution. Analysis of variance was performed for Ln(TMT), Ln(EC) and MY using the SAS package (1990) with a linear model including the following fixed effects: herd (1-4); milking session (1-3.706) nested within herd; stage of lactation (1-15); calving year (1-3); calving month (1-12); parity (1-3); classes of milking interval time (1-5); α linear regression coefficient of Ln(TMT) or Ln(EC) on milk yield per milking session. The variance components were estimated on a reduced data set (93,973 records relative to 362 lactations of 204 cows with all pedigree information available) according to a three-variate test-day animal model analysis using Pest-Vce software (Groenveld, 1998). The EC breeding values were calculated by univariate animal model analysis that included the following effects: milking session, parity, stage of lactation, calving month, the interactions between herd and stage of lactation and parity, permanent environmental effect of cow and additive genetic effect of animal. The EC breeding values were expressed on average 100±12 and compared with SCC-EBV estimates provided by Superbrown Consortium.

Results and conclusions

Descriptive statistics for traits considered are shown in Table 1. The mean value for EC are in agreement with results of Nielen *et al.* (1992) and Mucchetti *et al.* (1994). All the effects considered in the analysis of variance resulted statistically significants. Coefficients of determination (R^2) were 0.45, 0.17 and 0.57 for Ln(TMT), Ln(EC) and MY, respectively.

In Table 2 genetic parameters of traits of concern are given. The h^2 of milkability trait was equal to 0.31 and higher than value (0.13) estimated by Santus and Bagnato (1998) on Italian Brown cows. Heritability estimate for Ln(EC) appeared close to 0.23 and lower than value estimated by Goodling *et al.* (2000), on first lactation Holstein cows. Heritability of MY approached to 0.30. The repeatability values for Ln(TMT), Ln(EC) and MY were 0.69, 0.35 and 0.56, respectively. Linear regression analyses between EC and SCC genetic indexes showed a positive association (0.70), showing the potential rule of EC to predict sub-clinical mastitis.

In conclusion, given the moderate heritability value and the high correlation with SCC, electrical conductivity might be considered an useful trait in selection schemes for Brown Swiss cows with the aim, according to results of Norberg *et al.* (2004), of improving mastitis resistance and functional ability of dairy cows. Further studies based with more dairy herds should be needed to confirm these preliminary results, moreover, the estimation of economic value for EC and genetic correlations between EC with other production, type and

Table 2. Variance components estimates, heritabilities, repeatabilities and genetic correlations (rG) for total milking time (TMT), logarithm of TMT, electrical conductivity (EC), logarithm of EC and milk yield (MY).

	Ln(TMT)	Ln(EC)	MY
Genetic variance	0.019	0.003	2.072
Residual variance	0.019	0.010	2.771
Heritability (h^2)	0.312	0.226	0.331
Standard error of h^2	0.092	0.092	0.001
Repeatability	0.690	0.351	0.557
rG(S.E.) with Ln(TMT)		0.463(0.226)	0.749(0.210)
rG(S.E.) with Ln(EC)			0.552(0.113)

functional traits should be done before to introduce EC trait in an official selection index for Italian Brown cattle.

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FREE COMMUNICATION

Carcass traits of four rabbit genotypes

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ABSTRACT

Seventy-three rabbits of four genotypes (A - SIKA maternal line; C - SIKA sire line; AxC - hybrids between line A and C; AxCal - crossbreds between line A and the Californian breed) were used to evaluate the effect of genotype on carcass traits. Rabbits were weaned at 35 days and slaughtered at 93 days of age. Rabbits were fed standard feed mixture *ad libitum*. The highest live weight at slaughter and dressing percentage was achieved by line C, and the lowest in line A. Hybrids between line A and C exhibited slightly worse carcass traits than rabbits in line C, but the differences were not statistically significant. The Californian breed gave worse results than crossbreeding with line C, though in most cases the differences between AxC and AxCal were not significant. The differences between genotypes in hind leg tissue composition, pH and meat colour were not statistically significant.

Key words: Rabbits, Genotypes, Growth, Carcass quality

Introduction

Rabbit meat production is mainly based on two- or three-way line crossing or crossbreeding of highly fertile maternal lines or breeds with sire lines or breeds characterised with high growth rate and good carcass quality. In 1988 selection programme for maternal and in 1995 for sire line was started in Slovenia in Rabbit centre of Biotechnical Faculty. In sire line, the factors that influence growth rate, feed conversion ratio, slaughter, carcass and meat characteristics have to be controlled. The genetic factors have high effect on growth rate, proportions of separate digestive organs and carcass and meat quality (for review see Dalle Zotte, 2002). Selection on high growth rate can change carcass and meat quality as well (Piles *et al.*, 2000). The aim of the present study was to compare carcass traits of Slovenian sire and maternal lines, their hybrids and crossbreds between maternal line and Californian breed.

Material and methods

Seventy-three rabbits (42 females and 31 males) of four different genotypes were fattened after weaning at 35 days until slaughter at 93 days of age. Genotypes were: two lines of the Slovene SIKA meat rabbit, maternal line A (9 females and 8 males) and sire line C (7 females and 7 males), line crossing between A and C lines (8 females and 7 males) and crossbreds between line A and the Californian breed (18 females and 9 males). Animals were fed standard feed mixture (17% CP, 14% CF) *ad libitum*. Animals were fasted 2 hours before slaughter. Slaughter weight and warm carcass weight (excluding head and lower

Table 1. Carcass weight and composition of four rabbit genotypes (LSM ± SEE).

		Genotype			
		A	C	AxC	AxCal
Live weight	g	2754^{A}±60	3215^{B}±66	3094^{BC}±64	3001^{C}±49
Carcass weight warm	g	1384^{A}±33	1667^{B}±36	1585^{BC}±35	1520^{C}±27
Dressing percentage	%	50.24^{A}±0.38	51.83^{BC}±0.42	51.23^{AC}±0.40	50.66^{A}±0.31
Carcass weight cold	g	1360^{A}±33	1637^{B}±37	1555^{BC}±35	1494^{C}±27
Kidney fat	g	31^{A}±3	49^{B}±3	40^{BC}±3	$38A^{C}$±2
	%	2.30^{AC}±0.16	2.95^{B}±0.18	2.56^{BC}±0.17	$2.49A^{C}$±0.13
Fore quarter	%	26.91^{A}±0.25	27.25^{A}±0.26	27.59^{A}±0.26	27.05^{A}±0.20
Back	%	14.26^{A}±0.23	13.31^{B}±0.26	13.71^{AB}±0.25	13.25^{B}±0.20
Loin	%	20.45^{A}±0.28	20.77^{A}±0.31	20.72^{A}±0.30	20.97^{A}±0.23
Hind leg	%	36.19^{AB}±0.27	35.85^{AB}±0.30	35.53^{A}±0.29	36.36^{B}±0.22
Meat in hind leg	%	84.50^{A}±0.34	85.40^{A}±0.38	84.41^{A}±0.36	84.51^{A}±0.28
Bone in hind leg	%	15.03^{A}±0.31	14.55^{A}±0.34	15.02^{A}±0.33	15.10^{A}±0.25
Meat: bone ratio in hind leg		5.66^{A}±0.14	5.92^{A}±0.15	5.68^{A}±0.14	5.62^{A}±0.11

A - SIKA maternal line; C - SIKA sire line; AxC - hybrids between line A and C; AxCal - crossbreds between line A and the Californian breed;
^{A,B,C} *= P<0.05*

parts of legs, liver and kidneys) were recorded before and immediately after slaughter. Cold carcass weight was recorded 24 h after slaughter. After that kidney fat was removed, carcass was cut into fore and hind quarter between the 6th and 7th thoracic vertebra. Back was cut between last thorax and first lumbar vertebra. Hind leg was divided from loin between 6th and 7th lumbar vertebra. Right hind leg was further dissected into meat and bone. Percentage of cuts was calculated from cuts weight and cold carcass weight. Meat colour and pH were measured 24 hours after slaughter on the cross section between last thorax and first lumbar vertebra. Statistical analysis was performed by SAS statistical package (SAS, 1999) with GLM procedure. Effects of genotype (G_i) and sex (S_j) were included in the model. Interaction between genotype and sex was not statistical significant and was though not included in the model. Statistical model: $y_{ijk}=\mu+G_i+S_j+e_{ijk}$.

Results and conclusions

Carcass traits of rabbits of four genotypes are shown in Table 1. Sire line C reached the highest live weight at slaughter, while the maternal line A the lowest. The live weight of hybrid rabbits AxC differed significantly from rabbits in maternal line A but not from rabbits in sire line C. Live weight from crossbreds AxCal was slightly lower than from AxC and significantly lower than in line C. The highest dressing percentage was achieved by rabbits in line C, followed by hybrid rabbits AxC. Only the differences between lines A and C and between line C and crossbreeds AxCal were statistically significant. The highest kidney fat weight and percentage had rabbits from line C and the lowest from line A. Hybrid and crossbred rabbits were intermediate. Only the differences between line A and line C and between line C and crossbreds AxCal were significant. The differences in proportion of different carcass cuts were relatively small. There were no significant differences between different genotypes in percentage of fore quarter. Line A had significantly higher percentage of back than line C and crossbreds AxCal. The percentage of hind leg differed significantly only between hybrids AxC and crossbreds AxCal. The tissue composition of hind leg and the meat to bone ratio in hind leg did not differ between four genotypes.

Gomez *et al.* (1998) and Kermauner and Žgur (2002) reported differences in kidney fat and percentage of different cuts between different rabbit

Table 2. pH and meat colour of four rabbit genotypes (LSM ± SEE).

	Genotype			
	A	C	AxC	AxCal
pH 24	5.36±0.02	5.38±0.02	5.35±0.02	5.40±0.01
CIE L value	62.08±0.70	62.96±0.77	61.65±0.75	62.00±0.57
A* value	4.44±0.35	4.08±0.38	4.07±0.37	4.34±0.28
B* value	4.44±0.35	4.50±0.38	4.10±0.37	4.38±0.28

A - SIKA maternal line; C - SIKA sire line; AxC - hybrids between line A and C; AxCal - crossbreds between line A and the Californian breed;
There were no significant differences among four genotypes (P>0.05)

lines. Pla *et al.* (1996) also found differences in percentage of carcass cuts and kidney fat between two breeds differing in adult weight. Breeds with higher adult body weight had less fat at the same weight. Therefore Dalle Zotte and Ouhayoun (1998) reported the highest kidney fat percentage in line that reached the highest maturity at slaughter. In most experiments the adult weight is not known and so it is difficult to distinguish between effect of genotype and adult weight. In table 2 pH values and CIE L*, a* and b* values for meat colour are presented. pH 24 values indicated normal course of postmortal glycolysis. There were no significant differences among four genotypes, no in pH 24 values neither in meat colour.

Genetic differences in ultimate pH value and meat colour may reflect differences in muscle metabolism (Hulot and Ouhayoun, 1999). Dalle Zotte and Ouhayoun (1998) reported significant differences in L* and a* values between different genotypes, though the differences were very small. Pla *et al.* (1996) found only the differences in b* value on the surface muscle between different genotypes. Kermauner and Žgur (2002) did not find any differences in ultimate pH and meat colour between different genotypes. Between maternal line A and sire line C clear differences in carcass traits exists. Hybrids between both lines showed slightly worse carcass traits than rabbits in line C, but the differences were not significant. Crossbreeding with the Californian breed gave worse results than with line C, though in most cases the differences between AxC and AxCal were not significant. The genotype had no significant effect on rabbit pH and meat colour.

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FREE COMMUNICATION

Influence of the genetic origin and sex on live performance and carcass traits in the rabbit. Preliminary results

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ABSTRACT

The study compared the growth performance and carcass traits of 41 rabbits of both sexes derived from 3 genetic origins (GO): sire Vienna Blue (B), sire Burgundy Fawn (F) and hybrid rabbits (H). From weaning they were reared indoor in bicellular cages and fed *ad libitum* the same pelleted diet until a fixed slaughter weight (2.8±0.11kg). The rabbits of the 3 GO differed in slaughter age (88 *vs* 109 *vs* 122d for H, B and F-GO, respectively; P<0.001). Growth performance of B was better than that of F, while that of H was better than B+F groups. Carcass traits were not modified by the GO; only perirenal fat percentage increased from H (1.2%) to B (1.7%) to F (2.5% of the reference carcass; P<0.05) accordingly to the slaughter age. The sex effect was significant only for the dressing out percentage, being higher in males than females (59.0 *vs* 57.6%; P<0.05).

Key words: Rabbit, Genetic origin, Sex, Carcass composition

Introduction

Intensive rabbit meat production is based on hybrid rabbits, extracted from a few breeds and strongly selected for production traits. The hybrid rabbits are nowadays showing poor resistance to some diseases, which increases the use of chemicals. On the other hand, the current consumer is now looking for safer meats, obtained with the respect of the animal welfare and with good nutritional and organoleptic qualities. For these main reasons, the necessity for the utilization of rabbit breeds, purebred or their crosses, showing a good resistance to diseases is emerging, with the aim to reduce or stop the use of chemicals for the benefit of the consumer. In order to fit the farmer and the slaughterhouse needs, growth and feed efficiency together with carcass yield of the rabbits must be profitable. Among the adoptable breeds, Vienna Blue and Burgundy Fawn seem to be the more profitable to be introduced for this purpose. The aim of this study was to compare the live performance and the carcass yield of rabbits derived from 2 sire genetic origins, Vienna Blue and Burgundy Fawn, with the commercial hybrid rabbit (H group).

Material and methods

Forty-five weaned rabbits of both sexes were used. Fifteen of them were hybrid rabbits (H genetic origin - GO), 15 derived from Vienna Blue (B) and 15 from Burgundy Fawn (F) sire GO. The maternal genetic origin of B and F rabbits was a mixture of crosses of several medium-large size breeds, California and New Zealand White exclud-

Table 1. Growth performance of rabbits.

		Genetic Origin (GO)			Sex (S)		*P*-value			RMSE
		B	F	H	Female	Male	GO	S	GOxS	
N. rabbits		14	15	12	17	24				
Weaning age[1]	d	39	42	38	40	40				
Slaughter age	d	109[b]	122[c]	88[a]	106	107	***	ns	ns	9
Weaning weight	g	816[a]	790[a]	1079[b]	906	884	***	ns	ns	75
Final weight	g	2792	2737	2863	2810	2785	ns	ns	ns	113
ADG[2]	g/d	29.1[b]	24.4[a]	37.2[c]	30.1	30.4	***	ns	ns	3.5
Feed intake	g/d	152[b]	153[b]	140[a]	148	149	**	ns	*	9
FCI[3]		4.28[a]	5.02[b]	3.90[a]	4.41	4.39	**	ns	ns	0.58

*[1]Covariate; [2]Average daily gain; [3]Feed conversion index; *: P<0.05; ** or a, b: P<0.01; *** or a, b, c: P<0.001; GOxS interaction for feed intake, g/d: B= female 157.8, male 146.8 g/d (P<0.05); F=female 147.2, male 158.6 g/d (P<0.05); H=female 139.2 and male 141.2 g/d (ns).*

ed. All the animals were weaned at 40±4d and housed by pairs in cages in the same fattening rabbitry. A commercial pelleted diet was given *ad libitum*. Feed intake was recorded weekly. The animals were weighted at weaning and every week after the 11th week of age in order to find the prefixed slaughter weight of 2.8 kg.

Twelve H , 14 B and 15 F were then slaughtered at 2.8±0.11 kg and at 88, 109 and 122d of age, respectively. One B rabbit was excluded due to too light weight whereas 3 H rabbits because of they exceeded the prefixed slaughter weight. The chilled carcasses (CC) and the reference carcasses (RC = CC minus head, liver, kidneys, organs of chest and neck) were prepared as recommended by Blasco & Ouhayoun (1996). From the RC carcasses the perirenal fat and the other dissectible fat, the hind legs and the loin joint (1st–7th lumbar vertebra) were removed, weighed and expressed as %RC. One uncooked hind leg was furthermore dissected and its meat/bone ratio was calculated. ANOVA was performed using the proc GLM of the SAS (1990) program, by including the GO (H, B, F), the sex (S) and the GOxS interaction as fixed effects. LS means were calculated for all the effects involved in the model and the t test between means was calculated.

Results and conclusions

Table 1 summarises the effects of the GO (Vienna Blue: B; Burgundy Fawn: F; hybrid: H) and the sex (S) on the post-weaning growth. Although B and F rabbits were weaned at an elder age than the H rabbits, they were significantly lighter (816, 790 and 1079g, respectively; P<0.001) Subsequently, at the fixed slaughter weight of 2.8±0.11kg, the rabbits of the 3 GO significantly differed in the slaughter age (88 *vs* 109 *vs* 122d for H, B and F GO, respectively; P<0.001), indicating that B and F crossbred rabbits are less precocious and have lower growth performance than hybrid rabbits. Slaughter age, average daily gain, and feed conversion index of B-GO were better than those of F-GO (P<0.01). These results partly confirm those reported by Jensen and Tuxen (1982) referred on pure bred Vienna Blue and Burgundy Fawn rabbits. The growth performance were not influenced by the sex. The feed intake showed a GOxS interaction: in the rabbits of B-GO the feed intake of females were higher than that of males (157.8 *vs* 146.8 g/d; P<0.05) while in rabbits of F-GO the feed intake was reversed (147.2 *vs* 158.6 g/d; P<0.05); in the H-GO the feed intake was comparable. The effects of the GO and the S on the slaughter performance of rabbits are listed in Table 2. Carcass traits and slaughter yield were not significantly modified by the GO of the rabbits; only the perirenal fat percentage significantly (P<0.05) increased from 1.2% (H-GO) to 1.7% (B-GO) to 2.5% RC (F-GO) accordingly to the slaughter age. The 2 crossbred group of rabbits tended to

Table 2. Slaughter yield.

		Genetic Origin (GO)			Sex (S)		*P*-value			RMSE
		B	F	H	Female	Male	GO	S	GOxS	
N. rabbits		14	15	12	17	24				
Slaughter weight (SW)	g	2782	2705	2910	2812	2786	ns	ns	ns	111
Full digestive tract	%SW	16.6	17.7	18.7	18.3	17.1	ns	ns	ns	2.2
Skin and pawns	%SW	16.9	16.4	16.7	16.5	16.8	ns	ns	*	1.0
Cold dressing out	%	59.4	58.9	56.7	57.6	59.0	ns	*	ns	1.9
RC(1)	% CC(2)	81.6	81.4	82.2	81.8	81.7	ns	ns	ns	1.5
Perirenal fat	%RC	1.7^{a}	2.5^{b}	1.2^{a}	1.8	1.8	*	ns	ns	0.6
Total dissectible fat	%RC	2.6	3.6	2.5	2.9	2.9	ns	ns	ns	0.9
Hind legs	% RC	34.2	33.1	34.2	33.6	34.0	ns	ns	ns	1.0
Loin joint(3)	% RC	26.5$^{\beta}$	26.9$^{\beta}$	24.3$^{\alpha}$	25.9	25.8	0.06	ns	ns	1.5
Hind leg meat/bone ratio(4)		5.1	5.0	5.3	5.1	5.1	ns	ns	ns	0.6

(1)Reference carcass; (2)Chilled Carcass; (3)1st–7th lumbar vertebra; (4) determined on uncooked hindleg; α, β: P<0.1; or a, b: P<0.05; GOxS interaction for skin%SW: B= female 16.2%, male 17.6% (P<0.05); F= female 16.1%, male 16.7% (ns); H=female 17.2%, male 16.1% (ns).*

have an higher loin incidence than the hybrid group (26.5 *vs* 26.9 *vs* 24.3%RC, for B, F and H-GO, respectively; P<0.10). Contrarily to what found by Paci *et al.* (1995), the rabbits derived from Burgundy Fawn sire GO of the present work evidenced lower growth rate and worse feed conversion index. The sex effect was significant only for the dressing out percentage, resulted higher in males than in females (59.0 *vs* 57.6%; P<0.05). Also Paci *et al.*, (1995) observed that males belonging to Burgundy Fawn sire GO had higher dressing out percentages than females due to the significantly lower incidence of full digestive tract, as observed to a less extent (ns) in the present work. A GOxS interaction effect was observed on skin and pawn incidence: on B-GO the difference was significant (16.2%SW for females and 17.6%SW for males; P<0.05) whereas on the other 2 GO any sex-related difference was observed.

In conclusion, the live performance and the carcass quality of B-GO (sire Vienna Blue) are quite comparable to those obtained from the H-GO (hybrid). The animals of B-GO were 3 weeks elder than H rabbits, so, the extra cost due to the longer fattening period should be counterbalanced by the improvement of their meat quality. The meat quality traits are under evaluation.

Research supported by MIUR 2002 (PRIN - Prot. 2002078279). The farm "Azienda Agricola da Capeeto" V. Lago Dolfin, 2, Salboro 35124 – PD (Italy) is greatly acknowledged. The author wish to thank Emanuela Ragno for assistance in statistical analysis.

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FREE COMMUNICATION

Sheep internal parasites on Rab and Pag

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ABSTRACT

The purpose of our research was to determine which groups and species of internal parasites endanger the health of sheep on the islands of Rab and Pag. The research was carried out in 10 flocks on both islands taking the fresh dung out of 30% of the total number of sheep in each flock. It was ascertained that the gastrointestinal parasites and protozoa of Eimeria genus are present in most flocks on both islands. The presence of the fluke *Dicrocoelium dendriticum* was ascertained in considerably larger number of flocks on the island of Rab than on the island of Pag. On the other hand, the presence of parasites of *Moniezia* and *Nematodirus* genus was ascertained in larger number of flocks on the island of Pag. In two flocks on Rab parasites of *Protostrongylus* genus were ascertained while on the island of Pag they were not found in any flock.

Key words: Island, Sheep, Internal parasites

Introduction

According to the assessments of the Croatian livestock centre there is a population of over 100,000 sheep on the Adriatic islands. The sheep are being bred extensively in regions of rare vegetation typical for the Mediterranean. On pastures, the sheep are exposed to invasions of numerous parasites which affect the host pathogenically in various ways. In case of the balance between the carrier (sheep) and the parasite its pathogenic effect fails to take place. When the balance is disturbed diseases with clinical symptoms start to appear resulting in production dropping, and very often in the death of the animals. We assess the effect of parasites taking into consideration the influence of the environment, that is the way of breeding, climatic circumstances, age and number of animals in the flock. The islands of Rab and Pag are situated in the region of central Adriatic. On the island of Rab there is a population of 10,000 autochthonous sheep (the sheep of Rab) aiming at the production of lambs intended for slaughter. The average flock size is 20-40 head. On the island of Pag there is a population of 30,000 autochthonous sheep (the sheep of Pag) out of which 24,000 are being milked aiming at the production of the famous cheese of Pag, while the lambs are being slaughtered at the age of 28 days. The flock size is 40-100 (and more) sheep. The sheep are kept extensively during the whole year so they are permanently exposed to the invasions of numerous parasite species. The purpose of our research was to determine which groups and species of internal parasites endanger the health of sheep flocks on the islands of Rab and Pag.

Table 1. Internal parasites in sheep flocks on the island of Rab.

Type of parasite	Number of sheep in a flock										
	23	21	42	27	33	21	25	25	29	21	27
Gastrointestinal nematodes	+	+	+	+	+	+	+	+	+	+	+
Eimeria sp.	+	+	+	+	+	-	+	+	+	+	-
Protostrongylus sp.	+	-	-	+	-	-	-	-	-	-	-
Dicrocoelium dendrtiticum	-	+	+	+	+	+	+	+	+	+	+
Nematodirus sp.	-	-	-	-	+	-	+	-	-	-	-
Moniezia sp.	-	-	-	-	-	+	-	+	-	-	-
Trichuris sp.	-	-	-	-	-	-	-	-	-	-	+

Material and methods

The research was carried out in March of 2005 in 10 sheep flocks on both islands including the population of 269 sheep on the island of Rab, and 813 sheep on Pag. The number of sheep in a flock was from 21 to 42 head on Rab, and from 34 to 210 head on Pag. The sheep had been treated with anthelmintics agents in October and at the beginning of November. Clinical examination ascertained the state of sheep's health, and upon that the fresh dung of randomly selected 30% of the total number of sheep was collected together within the flock. The dung of each flock was deposited in marked plastic bags and transported to the parasitological laboratory for a coprology test. The test was made using the flotation method with $ZnSO_4$ (Boch and Supperer, 1986).

Results and conclusions

It was ascertained that all the sheep, in flocks included in the research, were clinically healthy. The results of coprology tests of the samples from the island of Rab are shown in table 1.

It is evident from the table 1, that on the island of Rab gastrointestinal nematodes are present in most flocks. They are followed by protozoa of Eimeria genus and the fluke *Dicrocoelium dendriticum*, while parasites of *Protostrongylus*, *Nematodirus*, Moniezia and *Trichuris* genus were ascertained in considerably smaller number of flocks. The results of coprology tests of the samples from the island of Pag are shown in table 2.

It is evident that on the island of Pag gastrointestinal nematodes are present in most flocks. They are followed by protozoa of *Eimeria* genus, endoparasites of *Moniezia* and *Nematodirus* genus, while the fluke *D. dendriticum* and *Trichuris* sp. were ascertained in smaller number of flocks. Comparing the sheep internal parasites' population on both islands it is evident that gastrointestinal parasites and protozoa of *Eimeria* genus were ascertained in almost every flock. Although *D. dendriticum* was ascertained in flocks on both islands it is noticeably more present in flocks on the island of Rab than on Pag. According to Džakula (1988) the invasions of *D. dendriticum*

Table 2. Internal parasites in sheep flocks on the island of Pag.

Type of parasite	Number of sheep in a flock										
	45	90	51	210	89	98	99	127	34	45	45
Gastrointestinal nematodes	+	+	+	+	+	+	+	+	+	+	+
Eimeria sp.	+	+	+	+	+	+	+	+	+	-	+
Protosrongylus sp.	-	-	-	-	-	-	-	-	-	-	-
Dicrocoelium dendrtiticum	-	-	+	+	+	-	-	-	-	-	-
Nematodirus sp.	-	+	-	-	-	-	+	+	+	+	+
Moniezia sp.	+	+	-	-	-	-	+	+	+	+	+
Trichuris sp.	+	-	-	-	-	-	-	-	-	-	+

were ascertained in rocky regions and islands of Cres and Brač, and the disease rarely has its clinical manifest. Rajković-Janje et al. (2000) ascertained the presence of *D. dendriticum* in 64% of sheep flocks in the region of Slavonia, therefore we can conclude that *D. dendriticum* exists in flocks in different climates. It is ascertained by this research that more flocks invaded by parasites of Moniezia genus reside on the island of Pag than on Rab. According to Canković (1988) and Jensen and Swift (1982) infestation with *Moniezia sp.* doesn't endanger so much the health of older sheep in comparison to lambs and younger sheep. However older sheep are polluting the pastures with eggs and therefore should be regularly treated with antiparasitics. In two flocks on the island of Rab parasites of *Protostrongylus* genus were also ascertained. On the island of Pag they do not exist. The presence of parasites of *Nematodirus* genus was ascertained in 6 flocks on Pag and in 2 on Rab. According to Jensen and Swift (1982) *Nematodirus sp.* doesn't make excessive damage in the sheep properly kept and nourished. According to Ceranić and Džakula (1988) and Jensen and Swift (1982) most parasites whose presence we ascertained on Rab and Pag mostly endanger the health of lambs and younger sheep, while with the older ones they caused clinical signs only in certain circumstances like underfeeding. On basis of the results of our research we can conclude that the sheep on the islands of Rab and Pag are comparably exposed to various groups and species of internal parasites that can considerably endanger the health of lambs and younger sheep in comparison to the older ones. Furthermore, we can conclude that the health protection preventive measures for sheep flocks on the islands of Rab and Pag should be adjusted to the results of coprology tests in order to choose the most effective way of parasites' prevention.

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FREE COMMUNICATION

Consumer satisfaction with Slavonian Kulen from Black Slavonian or modern pigs

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ABSTRACT

The goal of this research was to determine the consumers sensory acceptance of kulen made from Black Slavonian pigs and kulen of modern pig crosses and to find out how much particular sensory attributes influence overall satisfaction with kulen. The results showed that the consumer satisfaction with kulen from Black Slavonian pigs was the lowest compared to the satisfaction with kulen of modern pig crosses. The satisfaction with kulen taste had the greatest influence on overall satisfaction followed by its odour, colour and appearance.

Key words: Slavonian kulen, Consumers, Satisfaction, Sensory test

Introduction

One of the best-known original Croatian food products is Slavonian kulen - a dry sausage produced traditionally by many pig-breeding families in the region of Slavonia, east Croatia. It is produced from a mixture of minced lean pork from the most valuable cuts (ham, back, shoulder and neck), salt and spices such as minced pepper and garlic, and it is stuffed in pork blind gut. The sausage is naturally fermented, mildly smoked, slowly dried and left to mature for five months or more. The final product has high nutritive value and specific sensory characteristics concerning colour, odour and taste. Kulen is very appreciated and demanded in the Croatian market and it has high export potential (Kovačić *et al.*, 2003). In the past, Slavonian kulen was made principally from the local Black Slavonian pig. Nowadays it is produced mainly from the meat of more productive modern pigs and various crosses. However, with the current trends of protection of traditional foods the issue of originality of Slavonian kulen is of increasing concern. This actuated research of technological (Karolyi *et al.*, 2004) and economical (Juračak, 2004) comparison of Black Slavonian pigs with modern pigs used in the production of Slavonian kulen. Another important aspect in product development is consumer acceptance of a certain product. Many authors stated the importance of the sensory properties on food choice (Bogue and Ritson, 2003; Roeber *et al.*, 2002; Steenkamp, 1997). Sensory properties have a considerable impact in determining perceived quality (Caporale and Monteleone, 2003) and are very important factors which influence the liking or disliking of a product. The goal of this research was to compare the consumer sensory preferences towards kulen made from Black Slavonian pigs and kulen of modern pig crosses and to determine how much particular sensory attributes influence overall satisfaction with kulen.

Material and methods

The kulen sausages used for consumer testing were made at a meat product plant in Slavonia (PZ "Kulen Šokac", Drenovci, Croatia) following traditional manufacturing procedure. All batches of sausages were made by same recipe but each one with the meat from pigs with different genotypes. In total, batches of 104, 101, 129 and 116 kulens were manufactured from 10 Black Slavonian (BS), 10 Large White x Swedish Landrace (LWxSL), 8 Large White x Swedish Landrace sired with Duroc (LWxSL)xD and 12 Large White x Swedish Landrace sired with Black Slavonian pig (LWxSL)xBS, respectively. All pigs were raised on the same family farm under the similar housing and feeding regime and were between 16 and 18 months of age at the time of slaughter. During the manufacturing, sausages pass through same processing steps under the similar environmental conditions. At the time of testing all kulen samples were well matured (about 10 months). Consumers' preferences towards kulen were collected by means of a blind sensory test. The test was performed in September 2004, at two different manifestations; The "Kulen festival" that took place in Vinkovci (Slavonia) and the "Croatian farmers' products" in Zagreb. The overall satisfaction as well as satisfaction with 4 attributes (colour, taste, odour, and appearance) were measured on a 5-point Likert scale where 1 meant not satisfied at all, and 5 - very satisfied. Every respondent evaluated 2 different kulens which included 6 different sub-samples. The order of the first sample tasted was altered within subsamples. Kulen samples denoted as A, B, C and D originated from (LWxSL)xD, BS, LWxSL and (LWxSL)xBS pigs, respectively. Sample A was tasted by 120 respondents, sample B 123 respondents, sample C 121 respondents and sample D 124 respondents. Tested kulens were cut into small cubes (approximately 1x1x1cm) and served on a white plate. Additionally, the kulen half with its cross section was presented to the respondents for the evaluation of sausage colour and appearance. Respondents were asked to look, smell and finally to taste the kulen. They were allowed to take as many cubes as needed to make their judgements. Between two kulen samples respondents were offered bread and water. Univariate data analysis was performed to calculate consumers' satisfaction with tasted kulens. Nonparametric Mann-Whitney U was used to test the differences between different kulen samples. Multiple regression analysis was used to determine the relationship between overall satisfaction of kulen and the satisfaction with single kulen attributes. The stepwise selection method was applied to calculate regression coefficients, that is weightings for each partial satisfaction.

Results and conclusions

Out of 248 respondents, 155 were males. The average respondents' age was 45.65 (±14.0) years. The average evaluation of overall satisfaction and the average satisfaction with kulen attributes was calculated for each kulen sample (A-D). As it can be noticed from figure 1, kulen C and kulen D gained the same evaluation regarding overall satisfaction (4.13). The average value for kulen A was 3.80 and for kulen B only 3.41. Mann-Whitney test showed that the overall satisfaction with kulen C and D was significantly higher than the satisfaction with kulen samples A and B ($P<0.01$).

Regarding satisfaction with the tested kulen attributes, sample D achieved the highest scores for colour, odour and appearance, and it had the same score for taste as sample C. The less liked kulen for majority of respondents was kulen B produces from Black Slavonian pigs. No significant differences were found between consumers' satisfaction with kulen C and kulen D attributes. However, they are statistically significant different from kulen samples A and B ($P<0.05$). Sample A was also significantly higher evaluated than sample B regarding colour, odour and appearance.

Relationship between overall satisfaction and satisfaction with single kulen attributes

Overall satisfaction ($S_{overall}$) with kulen could be explained from partial satisfaction with their attributes. Using stepwise selection method all attributes were entered in the regression model: colour, taste, odour and appearance. Performed model explained 75% of variability in overall sat-

isfaction. Using regression coefficients we could formulate the model as follows:

$$S_{overall} = 0.302 + 0.387*S_{taste} + 0.198*S_{odour} + 0,168*S_{colour} + 0.165*S_{appearance}$$

As expected the relationship between overall satisfaction and partial satisfactions was positive. Satisfaction with kulen taste had the greatest influence on overall satisfaction. The second most important attribute was sausage odour. Kulen colour and appearance had very similar impact on the overall satisfaction. The acceptance of the kulen produced from Black Slavonian pigs by consumers was the lowest in the present research. However, at the present the quality of traditionally produced Slavonian kulen is not standardised and hence the sensory characteristics of final products may differ among producers, localities and seasons. Therefore, the present research should be repeated with more kulen samples from different producers in order to have more reliable indications about consumers' preferences. The results of this research give a first insight into consumer preferences regarding sensory characteristics of kulen sausage and the importance of different kulen attributes. Apart from the sensory properties of a product, food choice is also influences by the other information such as identity, origin, safety and nutritional properties (Caporale and Monteleone, 2003), as well as product name, package or label (Bárcenas *et al.*, 2001). Therefore, it is necessary not only to satisfy consumers' expectations regarding kulen sensory properties but also their expectations regarding other relevant kulen characteristics.

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CAPORALE

REVIEWERS AND EDITING ASSISTANT

The Editors wish to thank the following colleagues for their assistance in refereeing paper and posters submitted to the Symposium:

L. Bailoni; P. Berzaghi; S. Bovolenta; P. Carnier; M. Cassandro; C. Cavani; G.M. Chiericato; G. Cozzi; I. Curic; R. Dal Zotto; A. Dalle Zotte; L. Gallo; F. Gottardo; S. Gigli; R. Mantovani; M. Mele; C. Pieramati; M. Ramanzin; C. Rizzi; S. Segato; S. Schiavon; M. Spanghero; B. Stefanon; E. Sturaro; F. Tagliapietra; G. Xiccato; I. Zoccarato.

and the following PhD students for their assistance in the final editing of the papers:

O. Bonetti; L. Carraro; C. Ceolin; I. Cerchiaro; A. Comin; A. Corato; C. Dal Vit; M. De Marchi; L C.A. Elia;. Menegazzo; M. Noventa; C. Targhetta.