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Editors

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CONFERENCE ON THE "SUINO NERO SICILIANO (NERO SICILIANO PIG)"

Foreword

On behalf of the Scientific and Organising Committees, I have the honour and the pleasure to present the work and results of the 6th International Symposium on the Mediterranean Pig held in Messina – Capo d'Orlando in 2007.

The Symposium is returned in Italy after the third edition held in Benevento in 1995. As location, the Sicily was chosen, in particularly the area where the local breed "Nero Siciliano" is reared and processed in typical products. This area was a suitable location to represent the pig production in South Europe and the large participation of people at the Symposium underlined the rightness of this choice. Moreover, the symposium was characterized by a participation of delegates from Portugal, Spain, France, Italy, Slovenia and Croatia, showing an increasing common interest on the themes treated at the Symposium.

Following the "red line" that has connected all previous Symposia, the major arguments treated were the improvement and the management of the genetic resources, the sanitary approaches in the outdoor systems, the feeding and the rearing techniques, the quality of meat and meat products, the traceability for typical products and their socio-economical dynamics

The symposium gave also high concern to the pig's local breeds and to their meat products, highlighting the importance to preserve the biodiversity as well as the typicality of some unique pork products. The monitoring of pig parasitic diseases has been examined as well as the non conventional rearing systems used for typical pig breeds and their effects on the pork quality. It has been underlined also the importance of the products traceability and the need to better understand the purchasing dynamics of typical pork products.

In order to present as soon as possible the results of work done at the 6th International Symposium on the Mediterranean Pig, the Scientific Committee has decided to publish on line the proceedings to reach the widest audience possible. Due to the participation of a large scientific community, the volume includes 79 papers with a total of 448 pages. Even if this choice has led to a heavy effort, we are convinced that there is a need to provide timely and updated information on Mediterranean pig production that, while referring to an age-old tradition, must respond quickly to the challenges of the future.

About the future of Mediterranean pig production, I am optimistic because the renewed interest of farmers, retailers, consumers and policymakers on the sustainable development can not exclude the heritage represented by this production. I am optimistic also on the future of the Symposium. The University of Cordoba (Spain) has already offered to organise the forthcoming edition, and positive signals on the organisation of the future Symposia arrive from the Mediterranean Countries that have joined the European Community recently.

To conclude, I should like to underline the considerable amount of joint work carried out by the Organizing and the Scientific Committees that has made possible the realization of the 6th Symposium on the Mediterranean Pig. Thank of all of you.

Vincenzo Russo

SESSION 1
IMPROVEMENT AND MANAGEMENT OF THE GENETIC RESOURCES

ANALYSES OF THE EUROPEAN PIG DIVERSITY USING GENETIC MARKERS

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SUMMARY - An evaluation of the European pig diversity has been carried on by several countries with the support of the European Union over the period 1994-2000. This article presents an overview of the results of this investigation, focussing on two genetic marking techniques, namely microsatellites (MS) and Arbitrary Amplification of Fragment Length Polymorphism (AFLP). Nearly 200 loci were characterised on about 50 individuals from each of 59 to 71 breeds according to the marker considered. Sixteen local breeds belonging to four countries of the Mediterranean zone (Spain, France, Italy and Portugal) were involved in this research. The analysis of diversity based on genetic distances led to similar conclusion for the two markers (MS and AFLP), in spite of a markedly lower total diversity of AFLP compared to MS. The analysis of the MS loci showed that the allelic diversity pattern among breeds was quasi-independent from the diversity pattern based on allele frequencies. Genetic distances showed no particular clustering of local with international breeds, confirming the genetic uniqueness of the European local breeds compared to mainstream international breeds. The taxonomy of the local breeds studied revealed a cluster of the Spanish breeds of the Iberian type, in contrast with a wider dispersal of the breeds from other countries. Phylogeny often disagreed with documented breeds' history, showing the complex migration/admixture patterns which underlie the breeds' relationships. More generally, this work has been a source of methodological developments on various concepts related to genetic diversity evaluation. The database and the DNA depository created should also provide support for further innovative research in the field of domestic animal diversity management.

Key Words: Pig breeds, genetic marker, diversity, allelic richness

INTRODUCTION

The current state of the pig genetic resources in Europe is characterised by the existence of many local breeds, mostly rare, and a few intensively selected breeds of international status (e. g. Large White, Landrace, Piétrain, etc...). Such a situation makes it of particular interest to assess the level of genetic diversity that is present in Europe, in order to preserve genetic variation for traits likely to be the targets of current or future selection programmes. With this aim in mind, collaborative projects were launched with the support of the European Commission (EC) in the early 90s. A large set of European pig breeds was sampled and genetic markers used to assess diversity. The purpose of this paper is (i) to outline the history and scope of the *various projects*, (ii) to review the results obtained in the *partitioning of diversity* within and among the breeds sampled, (iii) to discuss the *relationships among breeds* evidenced, and (iv) the relevance of *molecular variation for quantitative trait diversity*, given the emphasis in this investigation on the exploitation of DNA marker information. Finally, *lessons and opportunities* offered will be briefly discussed.

EUROPEAN PROJECTS

Research on genetic polymorphisms in farm animals has a long history. For many years, it has been limited to blood groups, later followed by starch gel electrophoresis of proteins, so-called biochemical polymorphisms. A review of the pig genetic polymorphisms in the early 80s can be found in Ollivier and Sellier (1983). In the early 90s, DNA sequence variation started being intensively investigated and detailed porcine genetic maps were established, mainly including microsatellites but also allowing accurate mapping of several blood group and biochemical polymorphisms (Ollivier *et al*., 2001a).

The use of genetic markers for comparing breeds of pigs started in the late 60s (Major, 1968; Dinklage and Gruhn, 1969). It is also worth recalling the extensive literature devoted to comparing domestic breeds to wild pigs from various continents, with a view to tracing the possible origin of our present breeds. The shift of emphasis towards genetic diversity could only come with the development of efficient molecular genetic tools. A major contribution to the making of genetic maps was made through the Pig Gene Mapping Project (PiGMaP) supported by the EC over the period 1991-1996 (Archibald *et al*., 1995). In the second phase of this project, a pilot study on genetic diversity was undertaken along the recommendations made by a working group convened by FAO (Barker *et al*., 1993). The results obtained in this study, covering 18 microsatellite markers, were published by Laval *et al*. (2000).

Based on the experience gained in PiGMaP, a new project was launched by the EC in 1998, entitled *Characterisation of genetic variation in the European pig to facilitate the maintenance and exploitation of biodiversity* (in brief PigBioDiv). The main objective of PigBioDiv was to evaluate genetic diversity considering both commercial populations and local breeds, by providing the reference data necessary to estimate within-breed as well as between-breed genetic variability. This was achieved by sampling 50 individuals from each of 59 different breeds and lines and determining diversity at DNA level. The emphasis was on standard DNA marker technologies, such as *simple sequence repeat* (so-called microsatellites) and *arbitrary amplification of fragment length polymorphism* (AFLP), and on the use of high throughput genotyping devices (for details of the project see Ollivier *et al*., 2003, Groenen *et al*., 2003 and Plastow *et al*., 2003). The essential results can be found in SanCristobal et al. (2006) for microsatellites, Foulley *et al*. (2006) for AFLP, and Ollivier *et al*. (2005) for an overall analysis of genetic diversity, cumulating microsatellite (PiGMaP and PigBioDiv breeds) and AFLP (only PigBioDiv breeds) information. Some supplementary information can be obtained on the publicly available website:
http://www.projects.roslin.ac.uk/pigbiodiv/publications.html.

Table 1. European projects on pig biodiversity

Project (contract no)	Year	Number of countries	Number of breeds	Genetic markers (number of loci)	References
PiGMaP (BIO2-CT94-3044)	1994-1996	6	11	Microsatellites (18)	Laval *et al*., 2000
RESGEN (RESGEN-CT95-012)	1996-1998	6	19	Blood groups (13) Biochemical polymorphisms (11) Microsatellites (18)	Ollivier *et al*., 2001a
PigBioDiv1 (BIO4-CT98-0188)	1998-2000	15	59	Microsatellites (50), AFLP (148)	Ollivier *et al*., 2005 SanCristobal *et al*., 2006 Foulley *et al*., 2006
PigBioDiv2 (QLK5-CT-2002-01059)	2003-2006	China	45	Microsatellites, SNP, nuclear and mitochondrial genes	-

Pig genetic diversity evaluation was also included among the tasks of another EC-funded project entitled *European gene banking project for pig genetic resources*, in the framework of the EC regulation 1467-94 on genetic resources (RESGEN), over 1996-1998 (Ollivier *et al*., 2001b). This RESGEN project made use of some microsatellite results obtained in PiGMaP and PigBioDiv. More recently, a new EC pig biodiversity project was prepared shortly after the completion of PigBioDiv, including Chinese partners, with the intention of having the European experience extended to China. An overview of the main features of those European projects is presented in Table 1, which clearly shows an increasing coverage over time of both the resources and the genome of the species.

GENETIC AND ALLELIC DIVERSITY

In the analysis of diversity, a total of 70 breeds was available by combining the PiGMaP and PigBioDiv data (see Table 1). Excluding a sample of Wild Pig in PiGMaP and a sample of the Chinese breed Meishan in PigBioDiv, a subset of 68 European domestic breeds was finally analysed for both within-breed and between-breed diversity. These breeds belonged to three categories, namely local breeds, national varieties of international breeds and commercial lines from private breeding companies. Four countries of the Mediterranean zone participated by providing samples from 16 local breeds, listed in Table 2.

Table 2. Local breeds of the Mediterranean zone sampled in the European projects PiGMaP and PigBioDiv

Country	Breed name (PiGMaP breeds in bold)	Breed code	Sample size
Spain	Negro Canario	ESNC	18
"	Negro Iberico	ESNI	48
"	Manchado de Jabugo	ESMJ	36
"	Retinto	ESRE	68
France	**Basque**	FRBA	46
"	**Bayeux**[1]	FRBY	50
"	Créole (Guadeloupe)	FRCR	44
"	**Gascon**	FRGA	56
"	**Limousin**	FRLI	56
"	**Normand**	FRNO	52
Italy	Calabrese	ITCA	19
"	Cinta Senese	ITCS	30
"	Casertana	ITCT	28
"	Mora Romagnola[2]	ITMR	12
"	Nera Siciliana	ITNS	50
Portugal	Bisaro	PTBI	60

[1]Sample not considered in the PigBioDiv scientific papers
[2]Sample not individually typed

Genetic diversity within breed

The within-breed diversity has been analysed for AFLP and microsatellites. The average expected heterozygosity for each category of breed showed a similar tendency for both markers, namely lower within-breed diversity in local breeds and commercial lines as compared to international breeds. This observation appears to be in keeping with what is known of the average effective size of the breeds and lines of each category, though rather large variations appeared between populations of the same category. Care should therefore be taken when comparing individual breeds heterozygosities, given their rather large standard error of estimation. It should also be noted that heterozygosities expected under Hardy-Weinberg equilibrium were being considered, though this assumption could only be statistically tested for microsatellites. There it was rejected (at $P<0.05$[1]) for 15 breeds, which mostly showed a deficit of heterozygotes. Breed expected heterozygosities were converted into breed contributions to within-

[1] Not 0.01 given in SanCristobal *et al*. (2006)

breed diversity allowing a within-breed diversity breakdown over the three categories of breeds defined above (see CWs in Fig. 1). Contributions to within-breed diversity must add up to zero over breeds, and thus necessarily include some negative values, since the extinction of a highly homozygous breed raises the average heterozygosity of the remaining breeds.

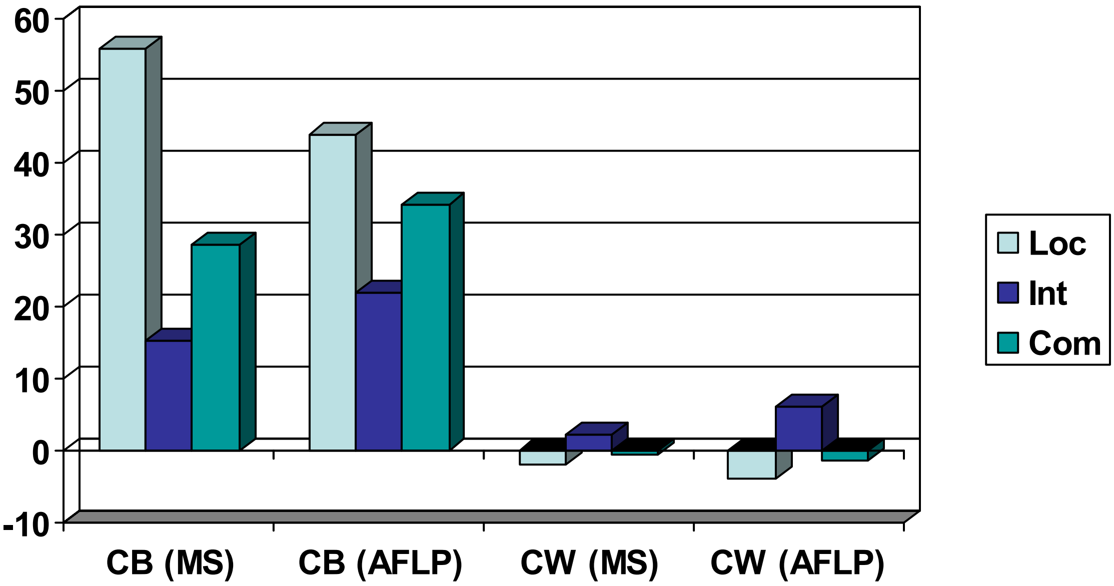

Figure 1 - Contribution (%) of each category of breed (Loc: local breed; Int: international breed; Com: commercial line) to between-breed diversity (CB), based on the Weitzman diversity function applied to Reynolds (Reynolds *et al*., 1983) genetic distances, and within-breed diversity (CW), based on expected heterozygosity.

MS: microsatellites on 68 European domestic breeds
AFLP: Amplified Fragment Length Polymorphism on 58 European domestic breeds
Adapted from Ollivier *et al*. (2005)

Genetic diversity between breeds

When analysing between-breed diversity, individual breed contributions to diversity may be derived from any set of distances. In a context of species conservation, Weitzman (1992, 1993) showed how to derive a diversity function *V* from a set of genetic distances in order to evaluate the relative loss of diversity resulting from the extinction of any given species. This loss is taken to represent its contribution to genetic diversity. Weitzman also showed that the algorithm leading to *V* generates a rooted tree which may be interpreted as a taxonomic tree, whose branch lengths measure the diversity lost when the corresponding species goes extinct (see next section on taxonomy). The approach has been extended to the situation of livestock breeds diversity by Thaon d'Arnoldi *et al.* (1998), and a software has been developed in the framework of PigBioDiv for implementing the calculations, down to the drawing of the taxonomic tree (Derban *et al*., 2002, 2005).

This method has already been used in most farm animal species and shown to be helpful for setting conservation priorities among endangered breeds (see review in Ollivier and Foulley, 2008). Between-breed diversity was partitioned in that way among the 70 breeds of PigBioDiv and a breakdown among the three categories could thus be achieved and compared to the corresponding breakdown for within-breed diversity. As shown in Fig. 1, the breed categories ranked in a reverse order for between- compared to within-breed diversity, with higher differences between categories for the former. About half of the between-breed diversity could be assigned to the local breeds. Similar examples showing large contributions of “native” breeds have been reported in cattle and sheep (Tapio *et al*., 2006). As explained above, some within-breed contributions may be negative. In contrast, the diversity function of Weitzman is a monotonously increasing function of the number of breeds and cannot yield negative contributions.

Comparisons between markers

Microsatellites (MS) and AFLP are both numerous and dispersed over the pig genome, making them both suitable for biodiversity analyses. Overall genetic diversity in AFLP was considerably below MS: 0.12 *vs* 0.56 and 0.11 *vs* 0.23 respectively for expected heterozygosity and Wright fixation index F_{ST} (Foulley *et al.*, 2006). In spite of these differences in total diversity, the individual breed contributions to both diversities (within and between) were positively correlated between the two markers, which is confirmed by the similarity between the MS and AFLP graphs of Fig.1. The correlations (r=0.5), however, were moderate and somewhat lower than would be expected if the two markers' evolutions had been governed mainly by genetic drift. This suggests that the two markers may carry different diversity information (Foulley *et al.*, 2006). An illustration is provided by the Italian breeds of PigBioDiv, which show that the international breeds (DU LR and LW) contribute much more to AFLP than to microsatellite diversity, whereas the inverse appears for the local breeds (CA CS and CT).

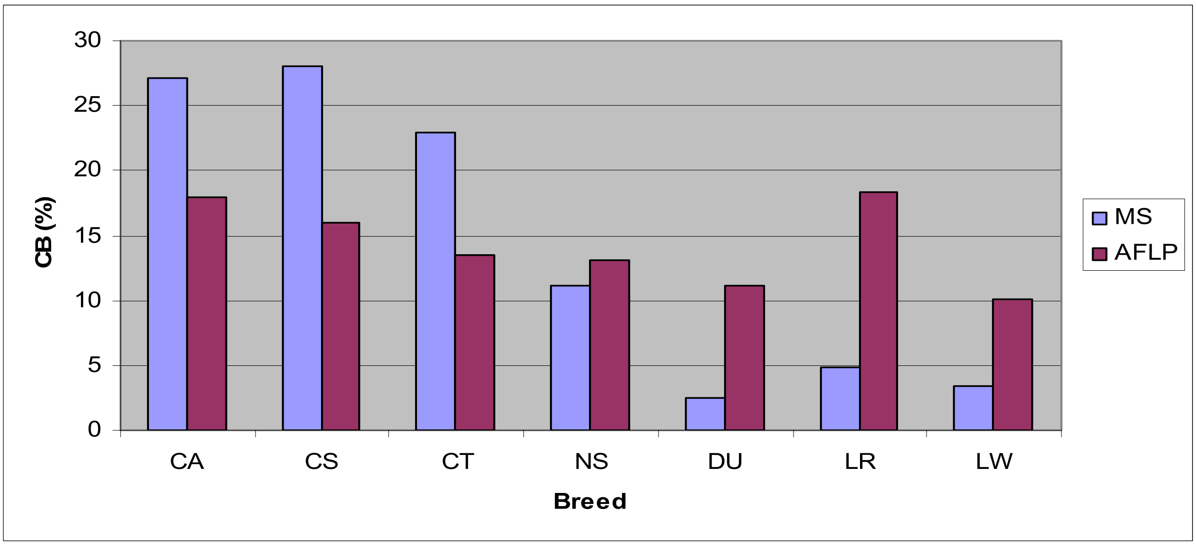

Figure 2. Relative contributions to between-breed diversity (CB defined as in Fig. 1) of 7 Italian breeds, expressed in % of the sum of their contributions to the European between-breed diversity.
Breed codes:
- CA, CS, CT, NS: see Table 2
- DU :Duroc ; LR: Landrace ; LW : Large White
MS: microsatellites
AFLP: Amplified Fragment Length Polymorphism

Allelic diversity

The number of alleles per locus, termed *allelic richness*, is a diversity measure of great interest in conservation genetics. While heterozygosity is related to the immediate response to selection, the long-term response is affected by the number of alleles (see the review of Barker, 2001). Marker allelic richness is also a useful criterion, as shown by the effectiveness of marker-assisted maximisation of the number of marker alleles conserved for retaining the maximum number of neutral and non-neutral alleles (Bataillon *et al.*, 1996).

The number of alleles observed in a breed sample depends on sample size (*N*). Fair comparisons between breeds then require equal sample sizes, or some way of correcting the number observed for sample size. Techniques used in ecology to study species diversity allow making fair comparisons, e. g. by applying the "*rarefaction"* method. The idea of rarefaction is to estimate allelic richness by the number of alleles expected in a sample of specified size, *g*, which is the smallest *N* of all breeds examined at a given locus (El Mousadik and Petit, 1996). Another possibility is to use an "*extrapolation"* method, proposed by Foulley and Ollivier (2006), who compared it to rarefaction on the PigBioDiv breeds.

The concept of allelic richness leads to the slightly different concept of *allelic diversity*, which refers to the existence of alleles specific to some breeds, since a high number of different alleles in a breed does not automatically guarantee their originality. The alleles present in one breed and absent in all others are called "private" alleles. Equivalently to the above definition of breed contribution to genetic diversity, the number of private alleles in a breed is a measure of its contribution to allelic diversity. This number has also to be corrected for sample size. This can be done either through rarefaction or extrapolation. An example is given in Fig. 3 which shows that the number corrected may considerably deviate from the observed number in either direction. Fig. 3 also shows that the Sicilian pig (NS) which harbours the largest number of private alleles is not among the highest contributing breeds to microsatellite diversity in Fig. 2. This example illustrates the need to distinguish allelic diversity, where allele uniqueness is at stake, from the classical genetic diversity concept, based on allele frequency. The quasi-independence found between the two types of diversity over the PigBioDiv breeds may apply to other species as well, as suggested by Foulley and Ollivier (2006).

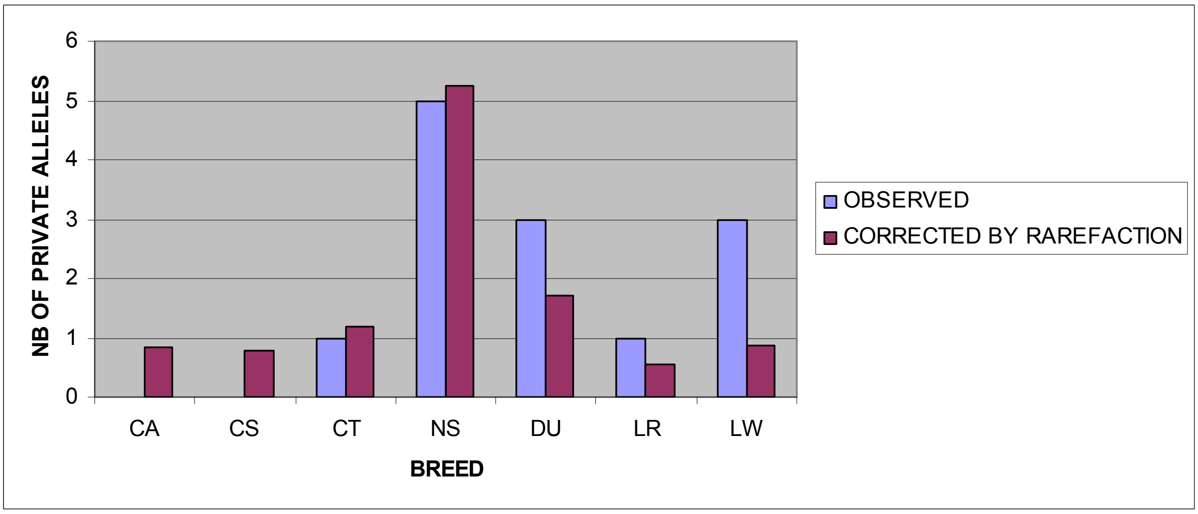

Figure 3 – Number of private alleles in 7 Italian breeds (breed codes given in Fig. 2): number of alleles "private" relative to the 7 breeds considered, totalled over 50 microsatellite loci.

TAXONOMY AND BREED CLUSTERING

In the previous section, it has been shown how genetic distances were used in PigBioDiv to analyse genetic diversity. Another classical use of genetic distances is the drawing of trees often termed phylogenetic trees. The term implicitly refers to evolution theory where diversity arises from speciation, i. e. the division of one ancestor species into two different species. Quite apart from the possible phylogenetic ambiguity of molecular data (as discussed for instance by Smouse, 1998), such a pattern of evolution can hardly applies to farm animal breeds, except in particular short-term situations when one breed (or line) happens to be subdivided into two new ones. Domestic breeds' evolution cannot in general be viewed as the result of a tree-like branching process. The trees drawn must be considered as telling the evolutionary story that best fits the diversity observed but not necessarily as telling the "true" story (Weitzman, 1992). Quite complex migration-admixture patterns usually prevail and the tree drawn is best viewed as a classification tool, showing a taxonomy rather than a phylogeny. The trees drawn from the PigBioDiv microsatellite and AFLP data showed a typical clustering of the commercial lines around their respective international breed of reference, but no clustering of local breeds with international breeds (SanCristobal *et al.*, 2006; Foulley *et al.*, 2006), in agreement with the local breeds' "uniqueness" shown in Fig. 1.

The taxonomy of the 30 local breeds investigated in the European projects is given in Fig. 4. This is the rooted tree generated by applying the Weitzman diversity function to the subset of these 30 breeds (see previous section). The longest branch is that of the French Basque, the breed contributing most to European between-breed diversity. The graph also shows the non-additivity of individual breed contributions, since the joint contribution of GBGO and GBBK for example, represented by the abscissa of their node (about 0.3), is much less than the sum of their individual branch lengths.

Fig. 4 shows no marked geographical clustering of the British, French, German and Italian breeds, in contrast with the Iberian cluster evidenced, which could have been expected from the common origin of the Retinto (RE) and Negro Iberico (NI) breeds. These in fact may be seen as two strains of the Iberian breed. To be noted, however, is the distinctive position of the other two Spanish breeds, the Manchado de Jabugo (MJ) known to be somewhat apart from the Iberian group (Martinez *et al.*, 2000), and the Negro Canario (NC) for which there is evidence of some African connection (Juan-Vicente Delgado, personal communication).

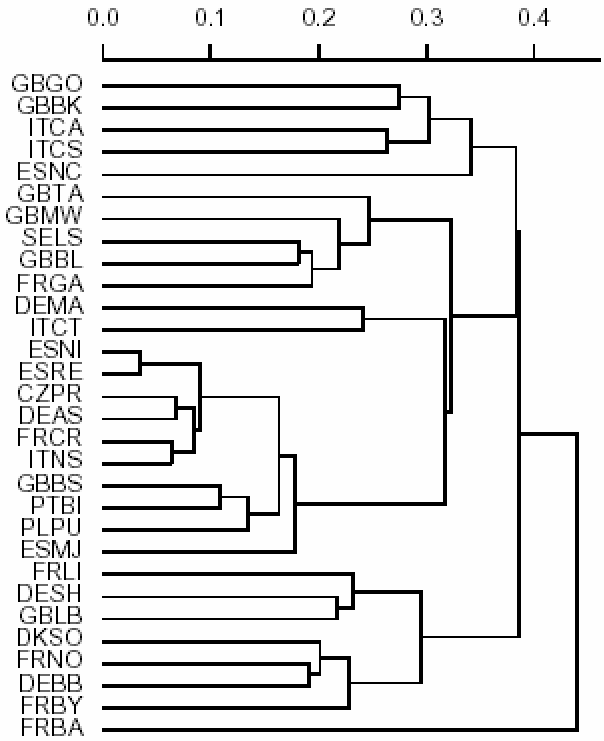

Fig. 4. Weitzman tree of the 30 local breeds of PiGMaP and PigBioDiv, based on Reynolds (Reynolds *et al.*, 1983) distances for microsatellites.

The breeds from Spain (ES), France (FR), Italy (IT) and Portugal (PT) are coded as shown in Table 2.
The other breeds are from:
-Czech Republic: CZPR, Presticke
-Germany: DEAS Angler Sattelschwein, DEBB Bunte Bentheimer, DEMA: Mangalica, DESH, Schwäbisch-Hällisches Schwein,
- Denmark: DKSO, Sortbroget,
- Poland: PLPU Pulawska,
- Sweden: SELS Linderödssvin,
- United Kingdom: GBBK, Berkshire, GBBL, British Lop, GBBS, British Saddleback, GBGO,
- Gloucester Old Spots, GBLB, Large Black, GBMW, Middle White, GBTA, Tamworth.

Particularly surprising is the close genetic vicinity of breeds originating from two islands as distant as Guadeloupe (FRCR) and Sicily (ITNS). It is also of interest to compare the microsatellite-based clustering of Fig. 4 with the history of the world pig breeds as reported in the popular handbook of Porter (1993). In several cases the documented history of “old” breeds is not supported by the clustering observed. The Créole pig (FRCR) for instance is quite far away from the Large Black (GBLB) reported as being among its main founding breeds. Similarly, the Polish Pulawska (PLPU) reportedly originating from a cross with Berkshire (GBBK) is quite distant from this breed. No trace appears to remain of the reported proximity of the Neapolitan pig, presently represented by the Italian Casertana (ITCT) breed, to several English breeds. This probably reflects the continuously blurred phylogeny of pig breeds as a consequence of complex migration-admixture patterns varying both in time and space.

MOLECULAR AND QUANTITATIVE TRAIT DIVERSITY

Breed diversity is probably the most useful information which can be drawn from a set of genetic distances in a context of conservation. This leaves open the question of the relevance of neutral marker diversity (anonymous) with regard to quantitative trait diversity (functional). One should, however, avoid concluding on the neutrality of the diversity measured from the supposed intrinsic neutrality of the markers used. We know that neutral genes can be affected by selection applied to neighbouring genes, a phenomenon known as *gene hitchhiking* (Maynard Smith and Haig, 1974). Selection acts on the whole genome and diversity is generated under the dynamics of multi-locus systems. This situation is in fact exploited, in reverse, in marker-assisted selection procedures using markers close to QTL (quantitative trait loci). Quite extensive QTL maps are now available for most farm animals including the pig. Due to their adequate coverage of the pig genome, most of the 50 microsatellites used in PigBioDiv have indeed been shown to be linked to a large number of quantitative traits (Ollivier and Foulley, 2008, from http://www.animalgenome.org/QTLdb). One would then expect to find some correlation between marker and quantitative trait diversity, particularly for those markers closely linked to QTL.

The testing of marker neutrality, however, is a challenging task. This is a field of evolutionary biology which has been extensively investigated for many years, and particularly with the recent advent of genome scans of DNA polymorphisms to elucidate the genetic basis of adaptive divergence in natural populations (reviewed, among others, by Storz, 2005). Similarly, the adaptation of domestic breeds to local conditions or to specific production objectives is expected to generate increased between-breed diversity and/or decreased within-breed diversity at those loci underlying the traits under selection and at nearby neutral marker loci.

Differentiation between populations as measured by F_{ST} is the basis of the test of selective neutrality proposed by Lewontin and Krakauer (LK) in 1973. The basic argument behind the LK test is that, under the null hypothesis of neutrality, differentiation at all loci should be the same. The observed variance of F_{ST} across marker loci can thus be tested against its expected value under the assumption of neutrality. Several improvements of the LK test have been proposed (reviewed by Ollivier and Foulley, 2008), essentially for taking into account the pattern of relationship among populations. Robertson’s (1975) prediction that any “structured” relationship will tend to increase the variance of F_{ST} has indeed been confirmed in PigBioDiv. For both microsatellites and AFLP a lesser departure from neutrality was observed by removing half of the breeds in order to approximate a star-like pattern of phylogeny (Foulley *et al*., 2006). Nevertheless, after correction for this effect, the LK test showed highly significant departures from neutrality for both markers, particularly large for AFLP.

The neutrality tests based on relative levels of diversity within populations exploit the reduction of variability around a selected locus due to hitchhiking, a phenomenon called “selective sweep”. Schlötterer *et al*. (1997) proposed a test based on the variance of repeat number at microsatellite loci. This test, however, may be sensitive to the demography-mutation model assumed. A more robust test can be performed by comparing groups of populations. The test statistic is the log of the ratio of variance in repeat number in two groups (Schlötterer, 2002). An application of this test to the PigBioDiv data showed the existence of "outlier" loci with contrasting allele size distributions between groups of breeds, indicative of selective sweeps (Ollivier and Foulley, 2008).

LESSONS AND OPPORTUNITIES OFFERED

PigBioDiv was one of the "demonstration projects" introduced in the life science and technologies programmes of EC as a "mechanism aimed at fostering the adoption of research results in real life practice" (Le Dour *et al*., 2000). PigBioDiv's objective was indeed to demonstrate the applicability of molecular biology tools for evaluating pig genetic diversity. Use was made of two standard marker technologies, and a wide spectrum of pig populations was examined. The activities pursued have been successful in making advances in the basic experimental design, operational modalities and analytical procedures for the broad-scale evaluation of animal genetic resources. The project has also demonstrated how effectively commercial and public sector entities and research staff can work together. Useful guidelines for future biodiversity projects were thus provided. Some prospects opened by further exploitation of the results will now be briefly discussed.

Methodology

This work has been a source of methodological developments on various classical concepts related to genetic diversity evaluation, such as Wright's fixation indices, genetic distances, and the Weitzman approach to diversity. In particular, the difficulties of analysis of recessive marker data such as AFLP have been thoroughly investigated. For that type of markers, Foulley *et al*. (2006) could recommend the moment-based approach of Hill and Weir (2004) instead of currently used procedures, in order to avoid potentially considerable biases in allele frequency and genetic diversity estimates. Further methodological developments can be foreseen for marker-based assignment and kinship estimation. Measuring genetic diversity in farm animals, however, still remains a challenge. Some insight into the multiple facets of this endeavour is given in the review of Ollivier and Foulley (2008).

Microsatellite technology

Individual genotyping

The advantages of microsatellites for evaluating diversity have now been known for a long time. Their abundance, wide dispersion over the genome, and highly automated characterisation make them a marker of choice. Difficulties, however, have been recognised in harmonising results from different laboratories, which requires standardisation of allele size. A coding system has been established in PigBioDiv, based on the mean and range of allele size compared to four control samples used in PiGMaP (Ollivier, 2002).

DNA pool genotyping

Microsatellite genotyping on DNA pools, known to be a cost-effective means to estimate allele frequencies, was also investigated in PigBioDiv. The need to select markers adapted to this technique (Groenen *et al*., 2003) and other technical difficulties restricted the typing to 20 out of the 50 markers used in individual typing. The technique is known to produce fluorescence peaks which are clearly artefacts, as confirmed in this study by the large excess of peaks compared to the number of alleles identified on the same breeds. Consequently, as shown in Table 3, expected heterozygosities were considerably larger and Reynolds distances lower, and both less variable, when based on peak frequencies observed on DNA pools compared to allele frequencies in individual samples from the same 22 breeds. The correlations in Table 3 also show that peak frequencies cannot provide accurate prediction of the standard diversity parameters. A statistical procedure to estimate allele frequencies (see for instance Skalski *et al*., 2006) is therefore needed in order to best exploit the information given by DNA pools.

A pig diversity database

The Roslin Institute was chosen as the ultimate data repository site in PigBioDiv. A database was mounted on the Roslin webserver and the data collected during the project were made available to the participants at http://www.databases.roslin.ac.uk/pigdbase/. Later on, a publicly available website was created at http://www.projects.roslin.ac.uk/pigbiodiv/publications.html, mentioned previously, for a wider dissemination of the PigBioDiv results, offering innovative opportunities to the pig industry (Roslin Institute, 2005).

Table 3. Comparison of expected heterozygosity and Reynolds (Reynolds *et al*., 1983) genetic distances obtained from individual (I) and pooled (P) DNA samples, typed for 20 microsatellites on 22 breeds (adapted from the PigBioDiv final report: Ollivier, 2002).

Parameter		**Expected heterozygosity**	**Reynolds distances**
Mean	**I**	0.55	0.24
	P	0.72	0.09
Standard deviation	**I**	0.05	0.08
	P	0.02	0.04
Range	**I**	0.46-0.66	0.06-0.45
	P	0.68-0.77	0.03-0.24
Correlation I-P		0.88	0.87

A DNA bank

During PigBioDiv, DNA has been collected over a set of 59 populations of pigs, originating from 13 European countries and including one Chinese breed. Part of this DNA has been stored in a duplicated DNA bank, at Roslin (UK) and Toulouse (France) in view of further research. The corresponding genotypes for 50 microsatellite and 148 AFLP loci are stored in the database previously described. Rules of access to this DNA have been defined in an agreement signed by the PigBioDiv parties, and put under the guidance of a specific committee (ConservPig Management Group) representing the interest of all parties [(2)]. This DNA depository, together with the database including the corresponding sample information, should provide support for further innovative research in the field of domestic animal diversity management.

[(2)] The committee is presently chaired by Dr Lawrence Alderson (http://www.lawrencealderson.com), former Chairman of Rare Breeds Survival Trust

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HETEROSIS FOR LITTER SIZE OF IBERIAN SOWS IS LIMITED TO SECOND AND LATER PARITIES

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SUMMARY – A datafile of 2,768 litter size records, from the 16 possible crosses among 193 sires and 817 dams of four strains of Iberian pigs, was analyzed in order to estimate heterosis effects and genetic parameters for prolificacy. A multitrait animal model was used to analyze as two different traits the number of piglets born alive in the first parity (NBA1) and in the second and later parities (NBA2+). Heritability estimates for NBA1 and NBA2+ were 0.16 ± 0.04 and 0.07 ± 0.02, respectively. Genetic correlation between both traits was estimated to be 0.74 ± 0.13. Moreover, heterosis effects on NBA1 and NBA2+ were estimated to be 0.10 ± 0.15 and 0.65 ± 0.10 piglets per litter, respectively. The different heritability and heterosis values and their moderate genetic correlation indicate that NBA1 and NBA2+ traits should be partially controlled, in Iberian pigs, by different genes.

Key words: Heterosis, diallel cross, litter size, Iberian pigs.

INTRODUCTION

Litter size is an important component of sow efficiency, and is therefore one of the traits included in the objective of many selection programmes of pig dam lines. Over the last two decades, selection for litter size has been successful to obtain moderate genetic changes for prolificacy despite of its low heritability (Petit *et al*., 1988; Estany and Sorensen, 1995; Noguera *et al*., 2002). Repeated records of litter size are available from each sow over different parities, and repeatability animal models are commonly used for its genetic evaluation. This model assumes that the litters farrowed in different parities are under the same genetic control, which implies complete genetic correlations between parities. Evidence of different additive genetic basis for litter size across the reproductive lifespan of the sow for different pig breeds has been reported (Irgang *et al*., 1994; Roehe and Kennedy, 1995; Noguera *et al*., 2002), specially at first and later parities (Serenius *et al*., 2003).

The knowledge of genetic parameters is the basis for successful selection and genetic improvement. In crossbreeding schemes, the expression of non-additive genes is even more important. Litter size is an important reproductive trait as it makes a major contribution to fitness. Generally, traits closely related to fitness show low heritability values because of the corrosive effects of directional natural selection on the additive genetic variance. So, major changes in litter size are mainly expected to come from the non-additive components of genetic variance. Heterosis and breed effects together are the primary genetic components of efficiency in pig crossbreeding systems. Heterosis effects are commonly explained by the presence of positive dominant genetic components. As previous works suggests litter size is partially controlled in Iberian pigs by different genes in the first and the successive parities (Fernández *et al*, 2008). Consequently, dominant components may also be different in sows and gilts.

Data from diallel cross designs, recorded on the N^2 possible crosses among N parental lines, allow to identify additive and non additive variance components of quantitative traits. The objective of this study was to investigate the genetic basis of litter size in Iberian pigs over the successive parities from data from a complete diallelic cross among four strains of Iberian pigs (Fernández *et al*., 2002).

MATERIAL AND METHODS

Datafile consisted on 2,768 litter size records from the 16 possible crosses among 193 sires and 817 dams of four ancient strains of Iberian pigs: *a*) *Ervideira*; *b*) *Campanario*; *c*) *Caldeira* and *d*) *Puebla*. The black hairless pigs of strains *Campanario* and *Puebla* proceed from Extremadura (Spain), and *Ervideira* and *Caldeira* are red strains from Alemtejo (Portugal).The size and structure of data and pedigree, and the mean and standard deviation of the analysed traits (NBA1: number of piglets born alive in the first parity, and NBA2+: number of piglets born alive in second and later parities), are presented in Table 1.

Table 1. Size and structure of data and pedigree, and main statistics of the analysed traits

Animals in pedigree	972
Sows with records	817
Litters	2768
In first parity	811
In second and later parities	1957
Season (S)	2
Crosses (TC)	16
NBA1, number born alive in first parity	6.20 (2.06)
NBA2+, number born alive in second and later parities	7.64 (2.27)

The number of sires and dams of each of the four strains, and the structure of the diallel crossbreeding scheme for the two parity classes considered is presented in Table 2.

Table 2. Number of litter size records for the two parity classes (first parity/later parities) and number of sires and dams of each strain used in a diallel crossbreeding scheme.

		Dam lines (No. of dams)				
Sire lines (No. of sires)		*a* (200)	*b* (183)	*c* (213)	*d* (221)	Total (817)
a	(56)	115/291	25/55	22/60	52/96	214/502
b	(39)	23/43	80/271	33/86	28/51	164/451
c	(50)	23/45	54/90	137/291	25/57	239/483
d	(48)	39/76	24/42	21/45	110/358	194/521
Total	(193)	200/455	183/458	213/482	215/562	811/1957

Litter records at the first and latter parities were treated as different traits. A bivariate analysis was performed using the VCE-5 software (Kovac and Groeneveld, 2003) to estimate (co)variance components, genetic correlation between traits, heterosis and breeding values of the sows. The model can be written in a general matrix form as follows:

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\end{bmatrix}=\begin{bmatrix}\mathbf{X}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{X}_2\end{bmatrix}\begin{bmatrix}\beta_1\\ \beta_2\end{bmatrix}+\begin{bmatrix}\mathbf{Z}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{Z}_2\end{bmatrix}\begin{bmatrix}\mathbf{u}_1\\ \mathbf{u}_2\end{bmatrix}+\begin{bmatrix}\mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{W}_2\end{bmatrix}\begin{bmatrix}\mathbf{0}\\ \mathbf{p}_2\end{bmatrix}+\begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix}$$

where the column vectors $\mathbf{y_1}$ and $\mathbf{y_2}$ represent the number of piglets born alive at first and latter parities of each sow (NBA1 and NBA2+); $\boldsymbol{\beta_1}$ and $\boldsymbol{\beta_2}$ are vectors of systematic effects on both traits, including the heterosis (2 levels: purebred and crosses) and season effects (2 levels), and the parity number (5 levels: 2 to 5 and ≥ 6) for NBA2+; $\mathbf{u_1}$ and $\mathbf{u_2}$ are vectors of additive genetic effects for each trait, $\mathbf{p_2}$ is the vector of permanent environmental effects for each sow with records in the second and latter parities; and $\mathbf{e_1}$ and $\mathbf{e_2}$ are vectors of random residuals. The incidence matrices $\mathbf{X_i}$, $\mathbf{Z_i}$, and $\mathbf{W_i}$ associate elements of $\boldsymbol{\beta_i}$, $\mathbf{u_i}$, and $\mathbf{p_i}$ with the records in $\mathbf{y_i}$ (i = 1, 2). The expectation of $\mathbf{y_i}$ is $\mathbf{X_i}\ \boldsymbol{\beta_i}$ and the variance-covariance structure of random effects of this bivariate animal model is as follows:

$$\mathbf{V}\begin{bmatrix}\mathbf{u_1}\\ \mathbf{u_2}\\ \mathbf{p_2}\\ \mathbf{e_1}\\ \mathbf{e_2}\end{bmatrix}=\begin{bmatrix}\mathbf{A}\sigma^2_{\mathbf{u_1}} & \mathbf{A}\sigma_{\mathbf{u_{12}}} & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{A}\sigma_{\mathbf{u_{12}}} & \mathbf{A}\sigma^2_{\mathbf{u_2}} & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma^2_{\mathbf{p_2}} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma^2_{\mathbf{e_1}} & \mathbf{I}\sigma_{\mathbf{e_{12}}}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_{\mathbf{e_{12}}} & \mathbf{I}\sigma^2_{\mathbf{e_2}}\end{bmatrix}$$

where **A** is the numerator relationship matrix; σ^2_{ui}, σ^2_{pi}, and σ^2_{ei} are variances of direct additive genetic, permanent environmental, and residual effects for trait i, respectively; σ_{u12} are the genetic covariances between both traits, and σ_{e12} their residual covariance.

RESULTS AND DISCUSSION

Phenotypic means and standard deviation for NBA1 and NBA2+ for each of the purebred and crosses of the complete diallelic scheme are shown in Table 3. As expected, the number of piglets born alive in the first parity, 6.20 (SD: 2.06), is lower than in second and successive parities, 7.64 (SD: 2.27). Previous works show that the number of piglets born alive in Iberian pigs increased up to the fourth- fifth parity, and then slowly decreased (Pérez-Enciso and Gianola (1992); Rodríguez *et al*., 1994; Fernández *et al*., 2008).

Table 3. Phenotypic means for the number of pigs born alive at first (NBA1) and second and latter parities (NBA2+) of the complete diallelic cross (SD between brackets).

Trait		*Dam lines*			
	Sire lines	(*a*)	(*b*)	(*c*)	(*d*)
NBA1					
	(*a*)	5.11 (2.41)	5.19 (1.77)	6.65 (2.04)	6.20 (1.91)
	(*b*)	5.23 (2.11)	5.87 (1.96)	6.66 (1.59)	5.97 (1.21)
	(*c*)	5.71 (2.23)	5.52 (1.74)	6.35 (2.06)	6.08 (2.07)
	(*d*)	5.51 (2.44)	5.77 (1.99)	6.39 (2.05)	5.97 (1.79)
NBA2+					
	(*a*)	7.00 (2.63)	7.65 (2.31)	7.70 (1.96)	7.64 (1.97)
	(*b*)	7.41 (2.79)	6.92 (2.12)	7.58 (1.98)	7.74 (2.08)
	(*c*)	8.10 (2.04)	7.66 (2.02)	6.96 (2.13)	6.88 (2.21)
	(*d*)	7.22 (2.74)	7.48 (1.97)	7.76 (1.84)	6.75 (2.20)

The different strains show important differences in litter size at first (NBA1) and second and latter parities (NBA2+) not only between the purebreds but also between crosses according to its line composition. Results from crossbred litters for NBA1 are in general slightly greater than those of purebred litters, being not significant the correspondent heterosis value (0.10 ± 0.15). On the contrary, the heterosis effect on NBA2+ is significantly different from zero, with an estimated value of 0.65 ± 0.10 piglets per litter (P<0.001). As is shown in Table 3, litter size in second and latter parities is always lower in purebred than in crossbred litters, with only one negligible exception (*c*x*c* > *c*x*d*). Heterosis for litter size in Iberian sows is then limited to second and later parities.

For NBA1, the means (and standard errors) of additive (σ_u^2) and residual (σ_e^2) variances were 0.69 (0.09) and 3.54 (0.09), respectively, being the heritability value h^2 = 0.16 (0.04). The corresponding values of variance components for NBA2+ were σ_u^2 = 0.36 (0.04) and σ_e^2 = 4.11 (0.05), being the permanent environmental variance σ_p^2 = 0.45 (0.05). The estimated values for NBA2+ of heritability, h^2 = 0.07 (0.02), and permanent environmental coefficient, p^2 = 0.09 (0.02), correspond to a value of repeatability, r^2 = 0.16. Homogeneous heritabilities and high values of genetic correlation (> 0.80, value considered as rule of thumb) would be expected if most of the genes affecting NBA at different parities were the same. Nevertheless, their different heritability values, as well as the estimated value of genetic correlation equal to 0.74 (0.13), confirm that NBA1 and NBA2+ traits are partially controlled by different genes. The genetic singularity of the litter size of primiparous sows has been evidenced previously not only in Iberian pigs (Fernández *et al*., 2008) but in other pig breeds (Hanenberg *et al*., 2001; Serenius *et al*., 2003).

Based on the results of this one and previous studies (Fernández *et al*., 2008), multiple-trait models should be recommended to estimate breeding values for litter size in Iberian pigs when first and later parity records are involved. A model for litter size treating NBA records at the first parity as one trait, and records at the later parities as one second trait could be more advisable than the repeatability model. Moreover, the heterosis for NBA2+ should be considered as a systematic effect in the model if litter data were available both from crosses within and between Iberian pig strains.

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GENETIC TRENDS FOR GROWTH OF YOUNG PIGS IN TWO BREEDING NUCLEI OF IBERIAN BREED

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SUMMARY – The genetic changes due to selection for growth performance during the postweaning period were evaluate in two breeding nuclei (BN) of Iberian pigs. The data files consisted of weight records between the 50 and 100 days of age from 1,325 and 3,859 pigs of BN1 and BN2, respectively. The statistical model assumed as different traits (weight at 90 days, W_{90d}) and daily growth rate (DG) the two parameters that describe the linear growth function (*a* = intercept, *b* = slope), and the analysis was carried out in a Bayesian framework via Gibbs sampling. The means of the posterior distributions of heritabilities, common litter environmental coefficients and genetic correlation in BN1 were 0.341 (h^2_a), 0.394 (h^2_b), 0.345 (c^2_a), 0.432 (c^2_b) and 0.772 (r_G). The respective values in BN2 were: 0.249 (h^2_a), 0.433 (h^2_b), 0.172 (c^2_a), 0.143 (c^2_b) and 0.761 (r_G). Positive genetic progress for growth was evidenced in both breeding nuclei, being the posterior mean of annual genetic trends for W_{90d}: 0.62 kg (BN1) and 0.18 kg (BN2), and for DG: 7.2 x10^{-3} kg/day (BN1) and 4.8x10^{-3} kg/day (BN2). The probabilities of these increments being greater than zero were 1.00 (BN1) and 0.99 (BN2) for W90d, and 0.99 (BN1) and 0.96 (BN2) for DG.

Key words: Iberian pigs, postweaning growth, Bayesian analysis.

INTRODUCTION

The methods to evaluate the genetic value of the breeding animals allow not only to select the best candidates for reproduction, but also to estimate the genetic changes achieved in a selected livestock population. In this sense, Bayesian techniques allow to analyze growth data taking into account the unequal information recorded from each animal, its relatives and the individuals whose data are located in the same systematic effects. Pig growth performances can be modelled using production functions, described by a reduced number of parameters that, in some cases, can be considered as underlying biological variables related to breeding goals.

A Bayesian procedure implemented by Varona *et al.* (1997, 1998) that allows a joint analysis of parameters of a linear production function, (co)variance components, systematic effects and breeding values was used to evaluate the genetic changes due to selection for growth during the postweaning period in two breeding nuclei of Iberian pigs belonging to the Spanish Association of Iberian pig Breeders (AECERIBER).

MATERIAL AND METHODS

Data available for the analysis for each of the two breeding nuclei (BN1 and BN2) of Iberian pigs are presented in Table 1. The data consisted of 2,643 (BN1) and 7,718 (BN2) weight records between 50 and 100 days of age, collected between 1999 and 2006, from 1,325 and 3,859 pigs for BN1 and BN2, respectively. As a general rule, each animal is weighted twice: the first one after weaning, at an age of about 60 days, and the second one at about 90 days.

Table 1. Size and structure of data and pedigree for each breeding nuclei and main statistics of the analysed traits

	Breeding Nucleus 1	*Breeding Nucleus 2*
Animals in genealogy	1363	4094
Testing batches	14	22
Litters	275	640
Animals with growth records	1325	3859
Growth records	2643	7718
Weight at 90 days (W90d), kg (sd)	24.72 (5.78)	28.57 (4.64)
Daily gain (DG), kg/day (sd)	0.30 (0.11)	0.39 (0.15)

The Bayesian procedure described by Varona *et al.* (1997) to analyze performance data using production functions has been applied to these data. In the postweaning period analyzed, the assumed model of growth was a linear function with parameters *a* (intercept) and *b* (slope), that correspond to the weight at 90th day (W_{90d}) and the rate of daily growth (DG), respectively. The k^{th} weight of the j^{th} animal recorded on day x_{jk} (animal age minus 90 days) is considered as a sample from the normal distribution:

$$y_{jk} \mid a_j , b_j , \sigma^2_t \sim N (a_j + b_j x_{jk}, \sigma_t^2)$$

where σ_t^2 is the variance of performances given the production function parameters.

The analysis of the growth function parameters requires another kind of variables related to genetic and environmental relationships between the production functions of different animals. These sources of variation were represented in the present analysis by the following bivariate animal model

$$\omega = \mathbf{X}\,\beta + \mathbf{Z_1 u} + \mathbf{Z_2 c} + \mathbf{e}$$

where ω = matrix of values of intercept and slope (*a* and *b*) of order N x 2; $\beta = [\beta_a , \beta_b]$, $\mathbf{u} = [u_a , u_b]$, $\mathbf{c} = [c_a , c_b]$ and $\mathbf{e} = [e_a , e_b]$ are matrices of systematic, additive genetic, common litter environmental and residual effects for both parameters; and $\mathbf{X}$, $\mathbf{Z_1}$ and $\mathbf{Z_2}$ are known incidence matrices. The systematic effects considered in β were the sex and testing batches. The marginal posterior distribution of each parameters of interest, including the genetic progress scores, was determined using a Gibbs sampling algorithm. Details about all these distributions can be found in Varona et al. (1997) and Fernández et al. (2002). The convergence was assessed by the double-chain method (García Cortés et al., 1998). The length of the Gibbs sampling was 550,000 discarding the first 50,000 (warm-up) and saving one sample from each 50 iterations.

Due to the lack of genealogical connections between both breeding nuclei, all the analysis were done independently. Two scores of genetic progress based on the average of the breeding values of the animals born in each testing batch were calculated: a) the total genetic trend, estimated as the difference between the average breeding values of the animals tested in the last and first batch, and b) the annual genetic trend, estimated rescaling the regression coefficients of the average breeding values on the temporal unit.

RESULTS AND DISCUSSION

The Bayesian procedure provides estimates for growth function parameters of each animal that take into account their own performances and the information from relatives and other unrelated animals sharing the same systematic effects (Varona et al., 1997). Moreover, this approach provides an estimation of the variance of performances given the production function parameters (σ^2_t), that includes errors in measure or physiological variations of the animals along the fattening period. The main statistics of σ^2_t and the variance ratios for the intercept (W_{90d}) and slope (DG) of the growth linear functions of the young pigs are summarized in Table 2.

The estimated values of heritability (h^2), both for W_{90d} and DG, were not significantly different between the two nuclei. Anyway, point estimates of h^2 and genetic correlations (r_u) were in the range of variation of the values obtained in related strains of Iberian pigs using this Bayesian procedure

(Rodrigáñez *et al*., 1999; Fernández *et al*., 2002). However, the estimated values of the common litter environmental coefficients (c^2) of the two traits were significantly different from zero according to the values of 95% highest posterior density intervals (95HPD), and were quite high. These results proves the convenience of include these effects in the analysis model for growth traits, even more when recordings are taken at early age (García Casco and Béjar, 1993).

Table 2. Means, posterior standard deviation (PSD) and 95% highest posterior density intervals (95HPD) of heritabilities (h^2), common litter environmental coefficients (c^2), and genetic (r_u), common environmental (r_c) and phenotypic (r_p) correlations between *a* (intercept = weight at 90 days; W_{90d}) and *b* (slope = rate of daily growth; DG) and variance component (σ^2_t) due to model fitting

	Breeding Nucleus 1			*Breeding Nucleus 2*		
	Mean	PSD	95%HPD	Mean	PSD	95%HPD
$h^2_{(W90d)}$	0.341	0.092	0.168 / 0.503	0.249	0.061	0.135 / 0.370
$h^2_{(DG)}$	0.394	0.098	0.223 / 0.576	0.433	0.082	0.258 / 0.581
$c^2_{(W90d)}$	0.345	0.039	0.269 / 0.420	0.172	0.018	0.136 / 0.207
$c^2_{(DG)}$	0.432	0.061	0.307 / 0.547	0.143	0.030	0.086 / 0.204
r_u	0.772	0.133	0.495 / 0.965	0.761	0.083	0.612 / 0.924
r_c	0.219	0.071	0.007 / 0.355	0.066	0.053	-0.004 / 0.169
r_p	0.579	0.043	0.493 / 0.660	0.742	0.022	0.698 / 0.786
σ^2_t	1.026	0.008	0.855 / 1.183	2.548	0.009	2.366 / 2.733

The use of Gibbs sampling also permits drawing of the marginal distribution of functions of estimated parameters, like the annual or total genetics trends, expressing its uncertainty (Blasco, 2001). Both nuclei shown significantly positive genetic progress for growth along the analysed periods (from autumn 1999 to autumn 2006 in BN1 and from autumn 2000 to autumn 2006 in BN2) as is shown in Fig 1. Thus, the total genetic trends for W_{90d} were 3.72 kg (S.D. 1.22) with values of 95%HPD interval (1.253 / 5.935) for BN1, and 1.28 kg (S.D. 0.51), with values of 95%HPD interval (0.349 / 2.328) for BN2. The probabilities of these increments being greater than zero were 1.00 for BN1 and 0.99 for BN2. The corresponding values for DG were 0.045 kg/day (S.D. 0.018) with values of 95%HPD interval (0.104 / 0.479) for BN1 and 0.022 kg/day (S.D. 0.014) with values of 95%HPD interval (0.000 / 0.048) for BN2, being 1.00 and 0.96 the respective probabilities of these increments being greater than zero.

The posterior mean and SD of the annual genetic trends for W_{90d} were 0.62 kg (S.D 0.20) and 0.18 kg (SD 0.08) for BN1 and BN2, being the respective values of the 95%HPD intervals equal to 0.207 / 0.959 and 0.022 / 0.349. The annual genetic trends for DG were 7.2×10^{-3} kg/day (S.D. 2.9×10^{-3}: BN1) and 4.8×10^{-3} kg/day (S.D. 2.9×10^{-3}: BN2), and only the bounds of the 95%HPD of BN2 include the zero value: 0.000 / 10.2×10^{-3}, being the same interval for BN1: 2.0×10^{-3} / 13.4×10^{-3}. However, the probabilities of these increments being greater than zero were 0.99 for BN1 and 0.96 for BN2.

An additional advantage of this method is that allows to estimated individual breeding values for 90 days weight as well as for average daily gain for animals having only one record. These animals must be excluded when analyzed through conventional methods.

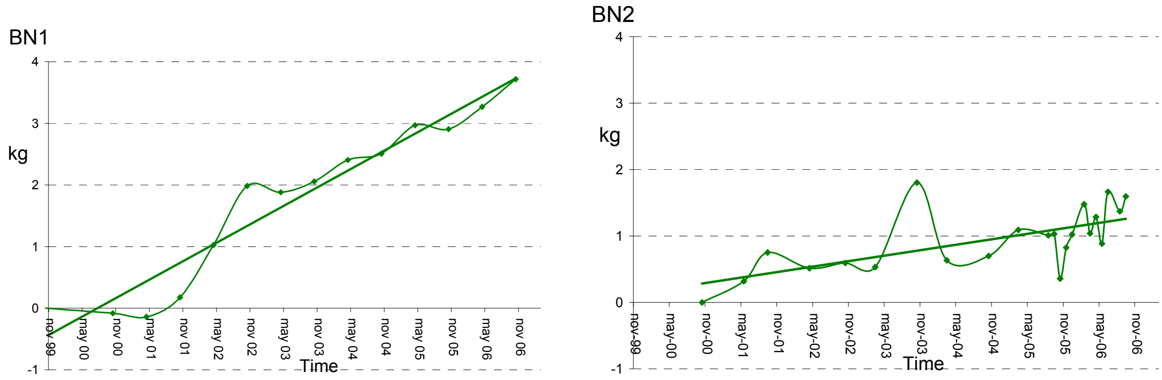

Fig. 1. Genetic trends for weight at 90 days in the two breeding nuclei.

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GENETIC CHARACTERISATION OF CELTIC-IBERIAN PIG BREEDS USING MICROSATELLITES

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SUMMARY - A genetic analysis of two Celtic-Iberian pig strains (Bísara and Asturcelta) using a set of 9 microsatellites (IGF1, S0005, S0090, S0155, S0218, S0225, SW240, SW632 and SW951) was carried out. Samples from Iberian (Alentejano and Iberian) and cosmopolitan pigs were used as reference. Between-breeds genetic kinship distance (Dk) varied from 0.357 (Alentejano-Iberian), to 0.480 (Bísara-Iberian). The Cosmoplitan pig has low Dk values with both Asturcelta and Bísara breeds (0.384 and 0.380, respectively) that have, in turn, a Dk value of 0.411. A neighbour-joining tree constructed on the between-individuals Dk distance matrix allowed identify 4 major clusters: i) Alentejano-Iberian; ii) Cosmopolitan; iii) Bísara; and iv) Asturcelta. The Celtic-Iberian individuals diverged from the Iberian samples at nuclear loci and were genetically closer to cosmopolitan pig. Within Celtic-Iberian strains genetic structure could result from population bottlenecks and reproductive isolation. This information may contribute to support conservation strategies for the Celtic-Iberian pig strains.

Keywords: Celtic-Iberian pig, microsatellites, phylogeny, kinship distance

INTRODUCTION

Native pig strains from the Iberian Peninsula have been classically divided in two different groups: 'Celtic' and 'Iberian' (Aparicio, 1944; Santos Silva et al., 2007). The major morphological differences existing between 'Celtic' and 'Iberian' (distributed mainly in the Southern Iberian Peninsula) pig strains have led to different genetic origins being hypothesized for each group: the Celtic pigs of the Iberian Peninsula would have a common origin with Northern-Central European pig breeds, whilst the Iberian pig breeds are assumed to be the pre-extant pigs in Iberia. Most Celtic-Iberian pig breeds are now extinct and the remaining Celtic-Iberian pig populations are extremely endangered. Nonetheless, some conservation efforts have arisen to preserve this genetic resource. In Portugal, a recovery programme has been initiated for the Bísara breed, which is mainly located in the Tras-Os-Montes area. In Spain, a recovery program has recently been initiated for the Asturian Celtic pig (Gochu Asturcelta). The aim of this communication is carrying out a preliminary analysis of two Celtic-Iberian pig strains (Bísara and Asturcelta) using microsatellites including also in the analyses samples belonging to Iberian pig strains (Alentejano and Iberian) and cosmopolitan European pigs as reference populations.

MATERIAL AND METHODS

Blood samples were obtained from 94 unrelated pigs belonging to 2 Celtic-Iberian (Gochu Asturcelta, As, 39; Bísara breed, Bi, 24), 2 Iberian (Alentejano ,Al, 13; Iberian, Ib, 8) and 3 cosmopolitan European pig breeds (Hampshire, Ha, 4; Landrace, La, 3; and Large White, LW, 3) breeds. Total DNA was isolated from blood samples following standard procedures (Sambrook et al.

1989). Individuals were genotyped with a set of 9 microsatellites (IGF1, S0005, S0090, S0155, S0218, S0225, SW240, SW632 and SW951) in an automatic sequencer ABI 310 (Applied Biosystems). Microsatellite information was analysed using the program MolKin 2.0 (Gutiérrez et al. 2005). Observed (Ho) and expected heterozygosity (He), raw (k) and rarefacted (k_n; Hurlbert 1971) average number of alleles per locus and molecular coancestry (*f*) (Caballero and Toro, 2002) were computed at the within-strain level to characterize genetic diversity in the analysed data set. The between-individual kinship distance matrix (D_k, Caballero and Toro, 2002) was also computed with the program MolKin and further used to construct a neighbour-joining tree using the program MEGA 3.1 (Kumar et al. 2004) or a bi-dimensional scaling plot using the statistical package SAS/SAT™ (1999). The kinship distance between two individuals *i* and *j* is calculated as $D_k = [(s_i + s_j)/2] - f_{ij}$, where s_i is the molecular coancestry of an individual *i* with itself or self-coancestry (which is related to the coefficient of inbreeding of an individual *i*, F_i by the formula $F_i = 2s_i - 1$) and f_{ij}, the molecular coancestry between two individuals *i* and *j*. Between strains D_k was obtained simply averaging the between pairs of individuals distances.

RESULTS AND DISCUSSION

Parameters characterizing the genetic diversity in the available microsatellite data set at strain level and for the whole data set are given in Table 1. The highest expected heterozygosities (roughly 0.6) is found for the Cosmopolitan strain whilst the lowest was found for the Alentejano individuals. This trend is also observed for the within-population f_{ij} which characterised the level of genetic identity of the individuals belonging to each breed. The average number of alleles per locus (k) is highly affected by sample size, and after rarefaction the Asturcelta breed showed a low value (2.8) for this parameter thus characterising the low number of reproductive individuals available at the beginning of the recovery program of the breed.

Table 1. Observed (Ho) and expected (He) heterozygosyty, molecular coancestry (f_{ij}) and raw (k) and rarefacted to eight copies (k_8) average number of alleles per locus for each strain analysed and for the whole data set.

Breed/strain	Ho	He	f_{ij}	k	k_8
Alentejano	0.410	0.414	0.575	3.0	2.3
Asturcelta	0.535	0.538	0.509	4.1	2.8
Bísara	0.473	0.579	0.475	4.3	3.1
Cosmopolitan pig	0.511	0.599	0.466	4.4	3.4
Iberian	0.470	0.557	0.510	4.3	3.3
TOTAL	0.893	0.626	0.382	7.2	3.4

Between-strains D_k varied from 0.357, for the pair Alentejano-Iberian, to 0.480, for the pair Bísara-Iberian. The Cosmoplitan pig has low D_k values with both Asturcelta and Bísara breeds (0.384 and 0.380, respectively) that have, in turn, a D_k value of 0.411. This overall situation is illustrated in Figure 1, which shows a bi-diemsional scaling plot constructed on the between strains D_k distance matrix. Dimension 1 on the X-axis differentiates the Iberian pig strains from the Celtic and Cosmopolitan pig strains whilst Dimension 2 on the Y-axis allows distinguishing between the Celtic-iberian pig strains with the Cosmopolitan pig located in an intermediate position.

This scenario was confirmed constructing a neighbour-joining tree on the between-individuals D_k distance matrix using the program MEGA 3.1 (Kumar et al. 2004). Four major clusters can be observed in Figure 2 including, respectively, the Alentejano and Iberian individuals, which clustered together and separated from the rest, the Cosmopolitan, the Bísara and the Asturcelta individuals. Three Celtic pig individuals clustered with the Iberian pig individuals. Two Asturcelta individuals clustered with the Cosmopolitan and the Bísara individuals and one Asturcelta individual clustered with the Bísara population.

The Celtic-Iberian strain samples had significant genetic divergence at nuclear loci with respect to the Iberian, samples, thus standing out as particular populations within the Iberian Peninsula. Moreover, Celtic-Iberian pigs are genetically closer to cosmopolitan (Northern-Central European) pigs than to the native Iberian strain. In any case, there is high genetic structure between the Celtic-Iberian strains probably due to the strong population bottleneck suffered during the second half of the 20th century and their reproductive isolation. Kinship distance is appropriate for analyzing populations of expected similar genetic origin and major reproductive isolation, as it does not exclusively reflect the recent genetic history of the taxa (Álvarez et al. 2005). In fact, D_k is closely related to Nei's minimum distance, though corrected for genetic similarity (f_{ij}) of the taxa before separation (Álvarez et al. 2005), thus allowing a good clustering of the strains analyzed here.

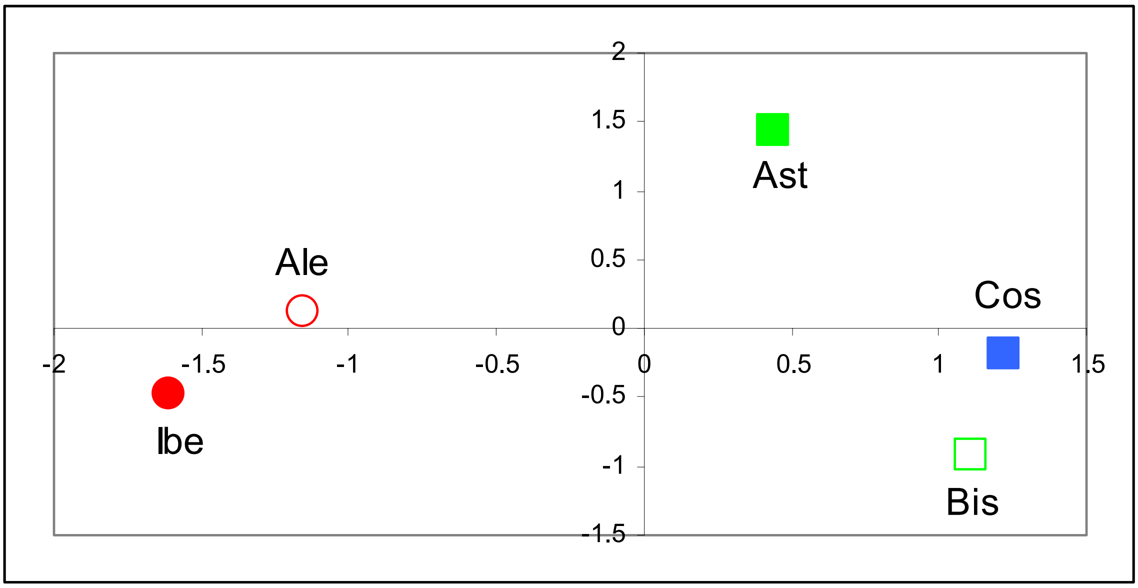

Fig. 1. Bi-dimesional scaling plot constructed on the between-strains Dk distance matrix. The Asturcelta, Bísara, Iberian and Alentejano breeds are represented, respectively, by a green square, an open green square, a red circle, and an open red circle, whilst the Cosmopolitan pig strain is represented by a blue square.

The information obtained in the present analysis may contribute to support conservation strategies for the Celtic pig strains native to the Iberian Peninsula. Celtic-Iberian pigs have restricted gene flow with other pig populations, probably because of different genetic origins and reproductive separation, and currently represent significant ecological and reproductive adaptive variation with respect the other Iberian strain of pigs or cosmopolitan pigs. Major efforts in the recovery of the breeds and the productive characterization of Celtic-Iberian pigs can be recommended.

ACKNOWLEDGEMENTS

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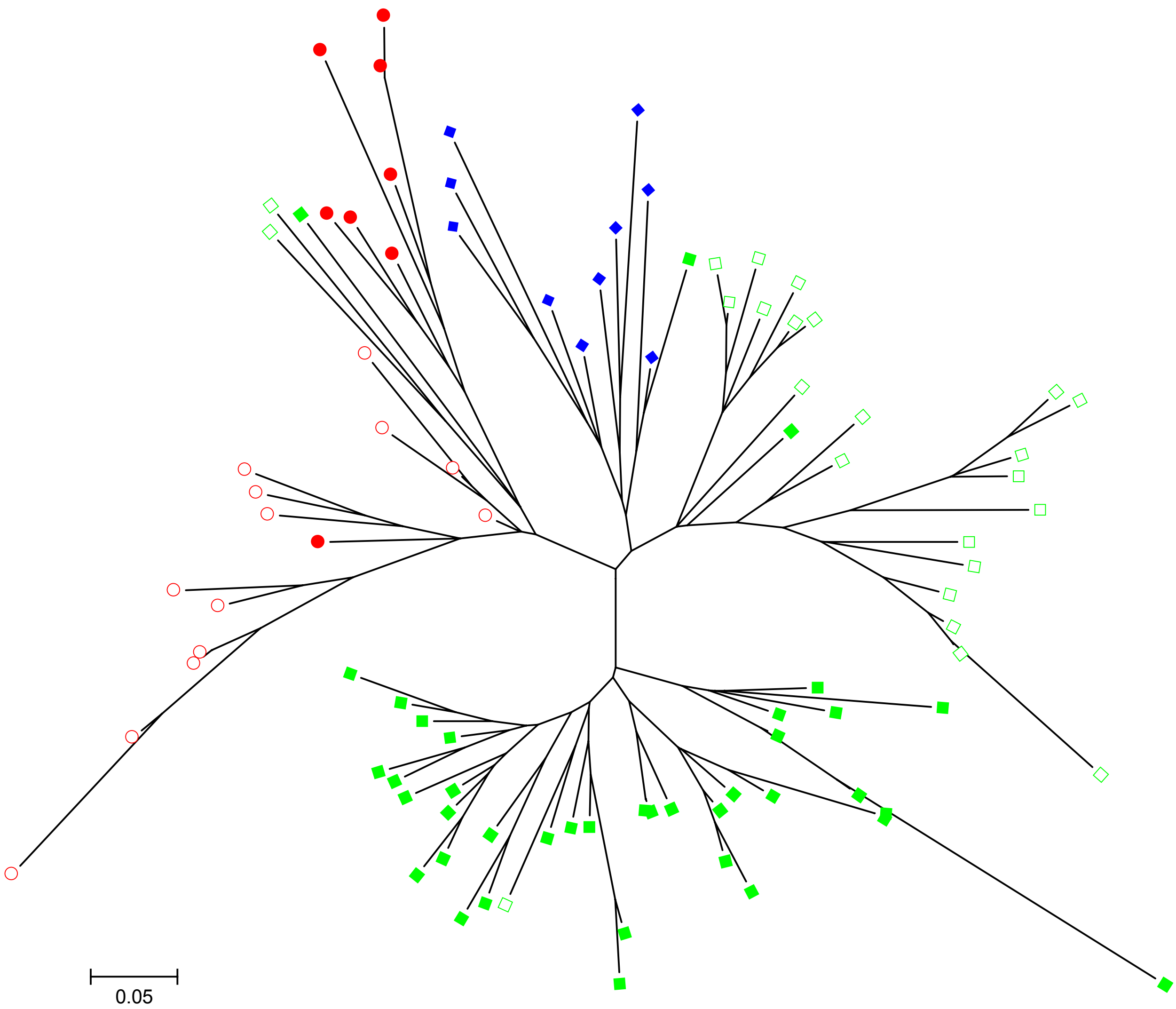

Figure 2. Neighbour-joining tree constructed on the between-individuals Dk distance matrix. The Asturcelta, Bísara, Iberian and Alentejano individuals are represented, respectively, by green squares, open green squares, red circles, and open red circles, whilst the Cosmopolitan individuals are in blue squares.

PEDIGREE ANALYSIS OF CINTA SENESE BREED

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SUMMARY - Cinta Senese is an autochthonous pig breed native of Tuscany. During last century the breed suffered a narrow bottleneck; in 1986 a census registered eighty-one sows and only three boars. The aim of the present paper was to assess the genetic contribution of the founders in order to conserve the genetic variability of the breed. Data analysis has been carried out with a set of Fortran packages. The genetic variability of the breed was estimated by pedigree information and by probabilities of gene origin. The percentage of known ancestors, the average generation interval, the average inbreeding coefficient, the number of founders and their marginal contribution were calculated. From the genetic point of view Cinta Senese breed can be considered as a small population. The results obtained showed how it is indispensable to continue the monitoring action of the breed. In order to control inbreeding some strategies could be suggested: choice of breeding animals "within family", use of planned mating strategies and counterbalance of founders' representation.

Key words: Cinta Senese pig, probability of gene origin, inbreeding, genetic diversity

INTRODUCTION

Maintaining genetic diversity is one of the main targets in the management of a population at risk and the rate of inbreeding is usually used to assess the loss of genetic variability. Nevertheless computation of individual and average inbreeding are strongly affected by the quality of genealogical information; incomplete pedigrees or introduction of new animals lead to underestimate the rate of inbreeding. In recent years some studies suggested other tools to evaluate genetic diversity.

Lacy (1989, 1995) introduced two new parameters: the "effective number of founders" and the "founder genome equivalents". The former measures the overall founder representation in a managed population regarding the loss of genetic variability due to unbalanced founder contributions whereas the latter represents the number of founders necessary to produce the same genetic diversity of the population under study if founders were equally represented. Previous approaches ignore potential bottlenecks in the pedigree, thus Boichard *et al.* (1997) proposed another parameter: the "effective number of ancestors" to account for bottlenecks in the population which are the major cause of gene loss in managed populations.

Cinta Senese is one of the most important Italian autochthonous pig breeds and, as other local breeds, suffered a bottleneck during the last century passing from 160,000 heads in 1950s (Raimondi, 1955) to 81 sows and 3 boars in 1986 (Gandini *et al.*, 2001). In 1996, a genetic management program started and a reduction of the inbreeding level of the animals (from 0.21 in 1995 to 0.15 in 2003) was obtained (Franci *et al.*, 2004). During the 1990s the population size increased reaching in 2006 the number of 1487 sows and 252 boars spread over 156 herds (Nardi, 2007). The national herd-book started in 1997 on the basis of recordings going back to the 1970s and Tuscany Region promoted a five year research program on the breed.

The aim of this work was to analyze the genetic variability of Cinta Senese pig breed to assess if it's still indispensable to continue working on inbreeding control and if it's possible to set up a plan of genetic improvement.

MATERIAL AND METHODS

We analyzed pedigree information registered in Cinta Senese herd book (source A.N.A.S.). A total of 13724 animals were registered (4807 males and 8917 females), spread out in 311 herds, mainly located in the province of Siena that is the most important with 37% of herds and 42% of animals. Pedigree completeness level was estimated by computing: number of full traced generations (g), where ancestors with unknown parents were considered as founders (generation 0); maximum number of generations traced; number of equivalent generations (g_e), that is the rate of known ancestors that reflects the completeness of the pedigree (MacCluer *et al.*, 1983) for each parental generation.

Furthermore the following parameters were also analyzed: individual and average inbreeding coefficient (F); average relatedness coefficient (AR) of each individual (AR can be interpreted as the representation of the animal in the whole pedigree regardless of the knowledge of its own pedigree); total number of founders (f); effective number of founders (f_e), defined as the number of equally contributing founders that could be expected to produce the same genetic diversity as that which exists in the population under study (James, 1972; Lacy, 1989); effective number of ancestors (fa); effective population size (Ne) defined as reported by Wright (1931); generation lengths computed for the 4 pathways (father-son, father-daughter, mother-son and mother-daughter). Parameters have been computed using the program Endog v 4.0 (Gutierrez and Goyache, 2005).

RESULTS AND DISCUSSION

Figure 1 shows the distribution of records, grouped in four-year periods (due to the scarcity of registrations years from 1972 to 1995 were represented all together). Only 6.5% of the individuals were registered before 1995, while most animals were registered from 2000, pointing out the population increment after the institution of the national herd book and the set aside of public funds to compensate the reduced profit to breeders. The last period includes only registrations up to May 2007 so the number of records appears lower.

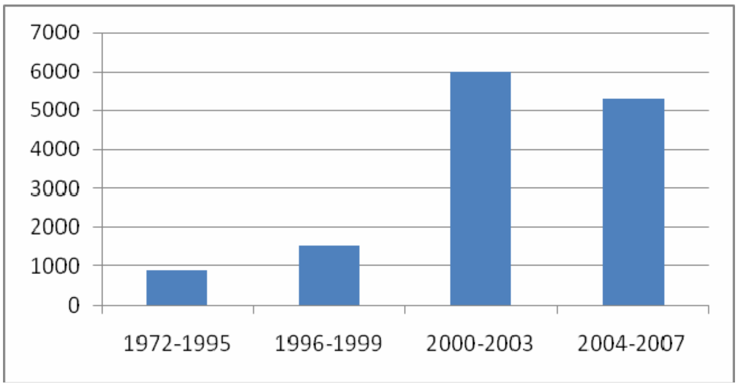

Figure 1. Herd book registrations from 1972 up to 2007.

Cinta Senese herd book is quite depth as confirmed by the rate of known ancestors per parental generation. The pedigree knowledge was higher than 90% for the first four generations of ancestors but it falls to 80% in the next parental generation and to 30% in the 8th generation (figure 2).

Average values of inbreeding (F) and relatedness (AR) are reported in table 1. The average inbreeding of whole population is 14,21%. This value is still too high indicating the importance of reducing the inbreeding coefficient through the exchange of breeding animals and avoiding mating between too much related animals. The mean average relatedness (AR) is strongly below the double of the inbreeding coefficient (F), indicating the lack of an adequate swap of breeding animals and the wide use of within-herd mating.

Figure 3 shows the trend of inbreeding computed by the year of birth of individual. Due to scarcity of data, average inbreeding is equal to zero for the first ten years; starting from the middle '80, with more genealogical data, inbreeding increases quickly and reaches a maximum peak in 1993 (0.23). After the institution of public incentive, the number of animals and of herds grew up fast and the average inbreeding coefficient kept to blow down.

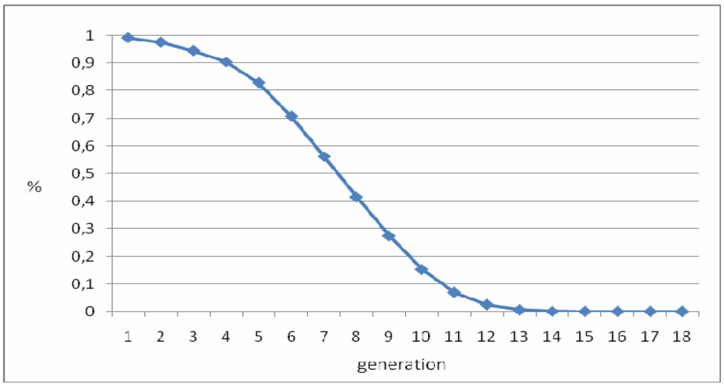

Figure 2. Rate of known ancestors.

Table 1. Inbreeding (F) and Average relatedness (AR) coefficients.

Number of animals	13724
Mean Inbreeding (F)	14.21%
Mean Average Relatedness (AR)	21.27%

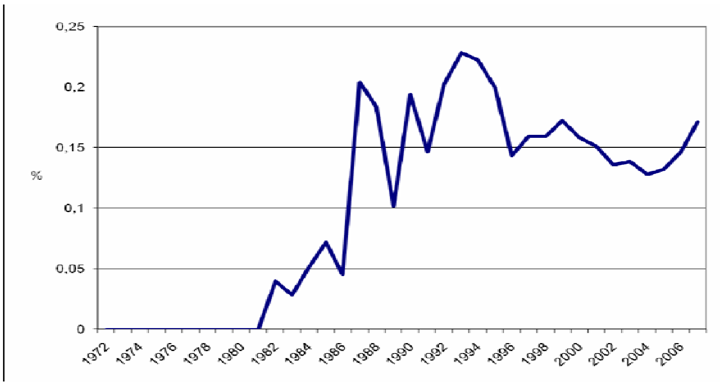

Figure 3. Inbreeding trend.

Table 2 reports the average generation intervals for the four pathways parent-offspring. The average generation length is 2.44 years; no significant differences were found between the four pathways. This result is comparable to previous studies performed on Cinta Senese pig breed (Baldi, 2004).

Table 2. Generation interval.

Pathway	N	Interval	STDEV	MSE
Father-Son	541	2.4942	1.4259	0.0613
Father-Daughter	2290	2.4423	1.2156	0.0523
Mother-Son	542	2.5643	1.4758	0.0635
Mother-Daughter	2289	2.4038	1.3580	0.0584
Average	5662	2.4434	1.3218	0.0176

Table 3 presents the effective population size estimated using two regression coefficients. The first one is the coefficient of the individual inbreeding over the maximum number of fully traced generations; while the second one is the regression coefficient of the individual inbreeding over the equivalent complete generations.

Table 3. Effective population size (N_e).

		ΔF	N_e
Mean Complete Generations	3.99	3.46	14.43
Mean Equivalent Generations	6.87	1.32	37.99

Figure 4 reports the trend of effective population size (Ne) estimated on the basis of family size variance. Computation of Ne based on the family size variance requires the knowledge of the average generation interval (2.44) that was rounded off to 2. Effective population size began to grow up with the demographic expansion of the breed, beginning from the end of the '90.

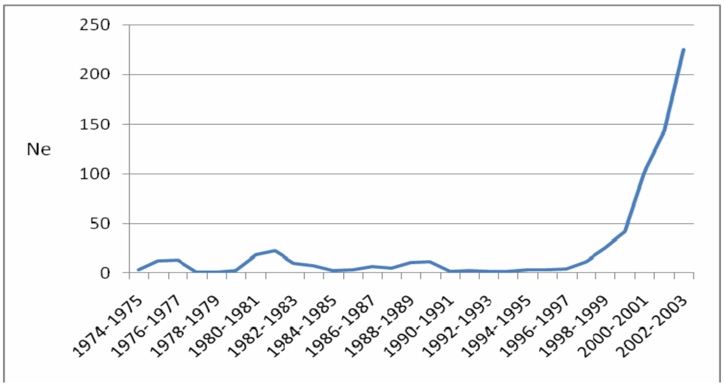

Figure 4. Trend of effective population size computed on family size variance.

Table 4 presents the total number of founders (f), the effective number of founders (fe) and the comparison between average inbreeding (computed) and the expected inbreeding produced by an unbalanced contribution of founders. At the beginning the Cinta Senese herd book was open, so the total number of founders is high; all animals with the right morphological characteristics were admitted, also those with unknown parents, so a lot of animals entered as founders but probably they were individuals with at least an unknown parents. At present the Cinta Senese herd book is closed, only animals with known parents already registered could be admitted.

Table 4. Effective population size (f_e).

Size of Population	13724
Total number of founders (f)	115
Actual Base Population (one unknown parent = half founder)	100.0
Effective Population Size of Founders (f_e)	10.99
Expected Inbreeding by unbalancing of founders contribution	4.55%.
Computed Mean Inbreeding	14.21%

Effective number of founders (f_e) is considerably lower (10.99) as a consequence of the scarce number of animals surviving in the '80 and of an unbalanced use of breeding animals. Only unbalanced contribution of founders produced 4.55% of inbreeding.

The effective number of ancestors (f_a) was computed using as reference population animals with both parents known (table 5). Total number of ancestors is 84, while the effective number of ancestors is quite similar to the effective number of founders, effects of past bottlenecks can't be evidenced because they occurred before the herd book reopening. Only four ancestors are necessary to explain more than 50 per cent of the genetic diversity of present population, as confirmation of the reduced number of animal from which the breed had been developed.

Both effective number of founders and effective number of ancestors are remarkably lower than values found in commercial pig populations (Baumung *et al.*, 2002; Maignel *et al.*, 2001), but are similar to some local French pig breeds (Maignel *et al.*, 2001).

Table 5. Effective number of ancestors (f_a).

Number of animals in the Reference Population	13609
Number of Ancestors contributing to the Reference Population	84
Effective Number of Ancestors for the Reference Population (f_a)	10
Nº of ancestors explaining 50%	4

CONCLUSIONS

The quality of pedigree information of Cinta Senese pig breed is quite high, considering the recent demographic history of the breed and the difficulty to obtain reliable paternities in an outdoor management system. Although the remarkably increase of the number of breeding animals, results show that inbreeding and average relatedness coefficient are still too high, so it is fundamental to carry on actions to control these genetic parameters. A high rate of inbreeding negatively influences reproductive traits and its effects are manifest in Cinta Senese breed (Crovetti *et al.*, 2005). The unbalanced contribution of founders appears clearly, so the maintenance of the genetic diversity of the breed is important to re-balance founders representation. Some strategies for genetic management could be suggested:

- the control of the inbreeding coefficient is fundamental to plan mating, choosing breeding animals with a relationship coefficient below the population average;
- the counterbalance of founders representation is desirable to identify descendants of less represented founders and to use them with higher intensity on population. Both objectives can be reached with a careful genetic management of the breed even if the lack of the artificial insemination makes these actions more difficult. Exchange of breeding animals would be supported by public and private breeders associations;
- to guarantee the maintenance of genetic diversity for the next generation a selection within family, and not among families, should be performed, choosing the best animal within each family and not absolute best animals. By this way every family will be represented in the next generation avoiding the risk that future breeding animals will be too much related. Selection criteria are only those reported on Cinta Senese herd book, and consist in respect of morphological standards, any other selection criterion (i.e. performance traits) is still to avoid with regard to inbreeding and relationship coefficients.

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STUDY OF GENES RELATED TO MEAT QUALITY IN CINTA SENESE PIG BREED

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SUMMARY - Cinta Senese is an autochthonous Tuscan pig breed, accounting for about 3,000 individuals characterized by good meat and fat quality. Several genes affect directly or are associated with meat quality traits and the causative mutations were identified in commercial pig breeds. This study investigated allele frequencies at some of these loci in Cinta Senese breed. DNA samples were analyzed by PCR-RFLPs at the following loci: ryanodine receptor 1 (RYR1) influencing mainly pH1 and responsible for the PSE defect in meat; calpastatin (CAST) affecting meat tenderness; protein kinase, AMP-activated, gamma 3 non-catalytic subunit (PRKAG3 or Rendement Napole locus) influencing muscle glycogen content, pHu and responsible for the defect known as acid meat; heart fatty acid-binding protein (H-FABP) related to intramuscular fat content and melanocortin 4 receptor (MC4R) associated with backfat thickness. The results for the RYR1 locus showed that the favourable allele (1843C) is almost fixed (0.98). All Cinta Senese animals were homozygous for the R200 allele of the PRKAG3 gene confirming the absence of the acid meat defect in the breed. Considering the low frequency of the 1843T allele for the RYR1 locus it will be feasible the eradication of the defective allele.

Key words: Cinta Senese, meat quality, PCR-RFLP, DNA markers.

INTRODUCTION

Cinta Senese is an autochthonous Tuscan pig breed, accounting for about 3000 individuals. The breed is characterized by high ability to live outdoor on chestnut and oak woods, low growth performances, good meat and fat quality and its commercial value is strongly linked to high quality seasoned products. Studies on commercial pig breeds identified mutations that affect directly or that are associated with meat quality traits. The aim of this project was to assess allele frequencies at these loci: RYR1, PRKAG3, MC4R, H-FABP and CAST. A mutation (C→T) at Ryanodine receptor 1 locus (RYR1, Fujii *et al.*, 1991) influencing mainly pH1 and it causes PSE syndrome (pale, soft and exudative meat), one of the major quality defect associated with abnormal post-mortem muscle acidification. PSE meat is characterised by a high acidification rate within the first hour after slaughter. PRKAG3 locus (protein kinase, AMP-activated, gamma 3 non-catalytic subunit, Meadus *et al.*, 2002), known also as RN locus, is associated with another meat defect called “acid meat”. A mutation (G→A) at codon 200 causes an excess of glycogen in muscle, that produces a prolonged and extreme drop of final pH. Both mutations produce a defective meat unsuitable for ham production. Calpastatin gene (CAST, Ernst *et al.*, 1998) is associated with post mortem meat pH values as well; several studies correlate some specific aplotypes at this locus with PSE phenotype. Cast gene is also involved in meat tenderness. A mutation (G→C) at MC4R (melanocortin-4-receptor, Kim *et al.*, 2000) locus is associated with backfat thickness; although in swine genetic selection for lean meat is required, in Cinta Senese fat cuts are also economically important. Finally H-FABP locus (Hearth fatty acid binding protein, Pang *et al.*, 2006) seems to be correlated with intramuscular fat content; previous studies at this locus performed on other pig breeds, show that some aplotypes could influence the IMF.

MATERIAL AND METHODS

DNA was extracted from hair roots and freeze-dried meat of Cinta Senese pigs, admitted to the herd book. All mutation points were identified with a PCR/RFLP analysis at the loci reported in Table 1. Sample represented from 1.3 to 6.6% of the whole population. At RYR1, PRKAG3 and MC4R loci were investigated the causative mutations, whereas at H-FABP and CAST loci only two of the three known polymorphisms were genotyped. Table 2 shows primer sequences and restriction enzymes used for the analysis and the number of individuals genotyped at each locus.

Table 1. Investigated loci, animals employed and annealing conditions

Locus	N. animals	Annealing temp.
RYR1	181	57°C
PRKAG3	129	57°C
MC4R	177	56°C
H-FABP	41	56°C
CAST	32	59°C

Table 2. Primer sequences, enzymes used in alleles determination at the investigated loci and number of individuals genotyped.

Locus	Primer forward (5'-3')	Primer reverse (5'-3')	Enzyme	n.
H-FABP	ATTGCTTCGGTGTGTTTGAG	TCAGGAATGGGAGTTATTGG	*Hae*III	60
			*Msp*I	41
CAST	GCGTGCTCATAAAGAAAAAGC	TGCAGATACACCAGTAACAG	*Hinf*I	38
			*Rsa*I	32
MC4R	TACCCTGACCATCTTGATTG	ATAGCAACAGATGATCTCTTTG	*Taq*I	229
PRKAG3	AAATGTGCAGACAAGGATCTCG	ACGAAGCTCTGCTTCTTGC	*Bsr*BI	129
RYR1	GTGCTGGATGTCTGTTTCCCT	CTGGTGACATAGTTGTGAGTTTG	*Hha*I	181

RESULTS AND DISCUSSION

Table 3 shows the number of genotyped animals at each locus and the frequencies of detected allele. The investigated polymorphisms presented similar frequencies (0.15 and 0.85 for alleles *D* or *d* and 0.93 and 0.07 for alleles *A* or *a*, respectively) at *H-FABP* locus. Concerning the *CAST* locus *A* and *F* alleles were both detected with 0.03 frequency. Whereas the *MC4R* locus showed intermediate frequencies for the two alleles (0.48 and 0.52 for allele *1* and *2,* respectively). All Cinta Senese individuals were homozygous for the rn^{+} allele at the *PRKAG3* gene (figure1) confirming the absence of the acid meat defect for this breed.

The results at *RYR1* locus (figure 2) show that the positive allele (*1843C*) is almost fixed (0.98). The tested animals were in H.W. equilibrium at all the examined loci, except for the first polymorphic site at the *H-FABP* locus that showed an excess of heterozygosity. Allele frequencies at RYR1 locus in Cinta Senese breed were similar to those reported for Duroc, Large White and Landrace pig breeds raised in Italy (Russo *et al.*, 1996) and the allele for halothane sensitivity seems almost totally eliminated in the breed. Similar results were found at PRKAG3 locus where the rn^{+} allele was fixed as reported for Large White pigs (Miller *et al.*, 2000). Cinta Senese presented similar frequencies for the two alleles at MC4R polymorphism as stated for Lithuanian White pigs (Jokubka *et al.*, 2006) whereas other authors (Stachowiak *et al.*, 2005) found substantial differences in allelic frequencies between Polish Large White and Landrace that may underlie intrabreed variability in production traits probably absent in Cinta Senese. The two investigated polymorphism at H-FABP gene presented allelic frequencies similar to those found for Large White and Landrace pigs (Gerbens *et al.*, 1997) with a prevalence of the A (*MspI*) and D (*HaeIII*) alleles whereas various Chinese breeds were found homozygous at these alleles (Pang *et al.*, 2006). Only the Duroc breed differs substantially at these loci (Gerbens *et al.*, 1997; Pang *et al.,* 2006) showing intermediate frequencies. Observing the whole results at the studied polymorphisms Cinta Senese seems to be quite similar to Large White breed even if some differences emerged which could be ascribed to the different selection pressure applied in the two breeds.

Table 3. Genotypes and allele frequencies observed at the investigated loci

Locus	Genotypes			Allele frequencies	
H-FABP (*Hae*III)	D D	D d	d d	D	d
	2	14	44	0.15	0.85
H-FABP (*Msp*I)	A A	A a	a a	A	a
	37	2	2	0.93	0.07
CAST (*Hinf*I)	A A	A B	B B	A	B
	0	2	36	0.03	0.97
CAST (*Rsa*I)	E E	E F	F F	E	F
	30	2	0	0.97	0.03
MC4R (*Taq*I)	1 1	1 2	2 2	1	2
	35	99	43	0.48	0.52
PRKAG3 (*Bsr*BI)	RN^{-} RN^{-}	RN^{-} rn^{+}	rn^{+} rn^{+}	RN^{-}	rn^{+}
	0	0	129	0.00	1.00
RYR1 (*Hha*I)	1843C 1843C	1843C 1843T	1843T 1843T	1843C	1843T
	180	7	0	0.98	0.02

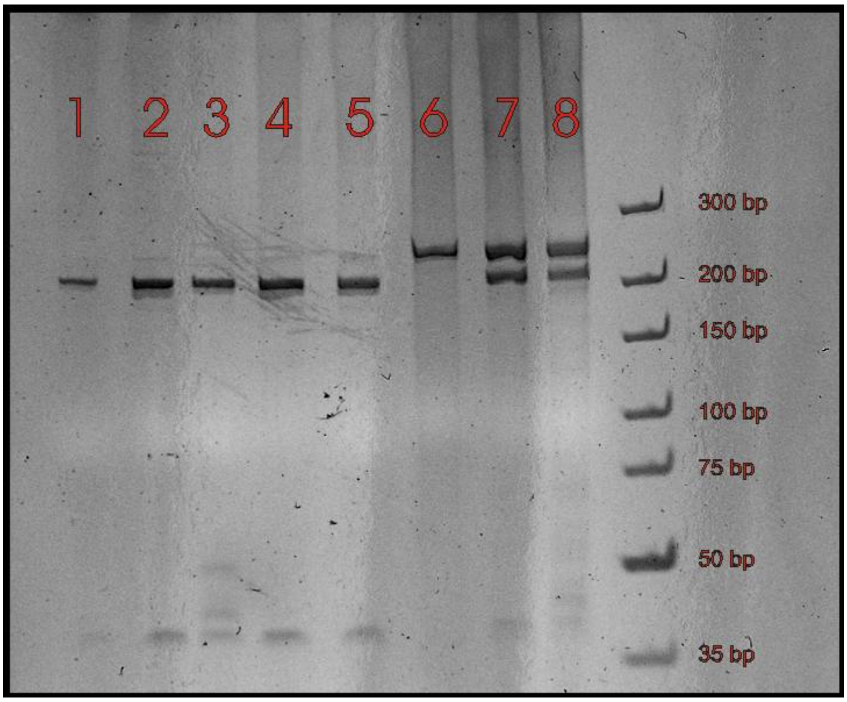

Figure 1. PCR-RFLP at *PRKAG3* locus. Lanes 1, 2, 3, 4 and 5: Cinta Senese ($rn^{+}rn^{+}$); Lane 6 (RN^{-} RN^{-}) and lanes 7 and 8 (rn^{+} RN^{-}): Hampshire.

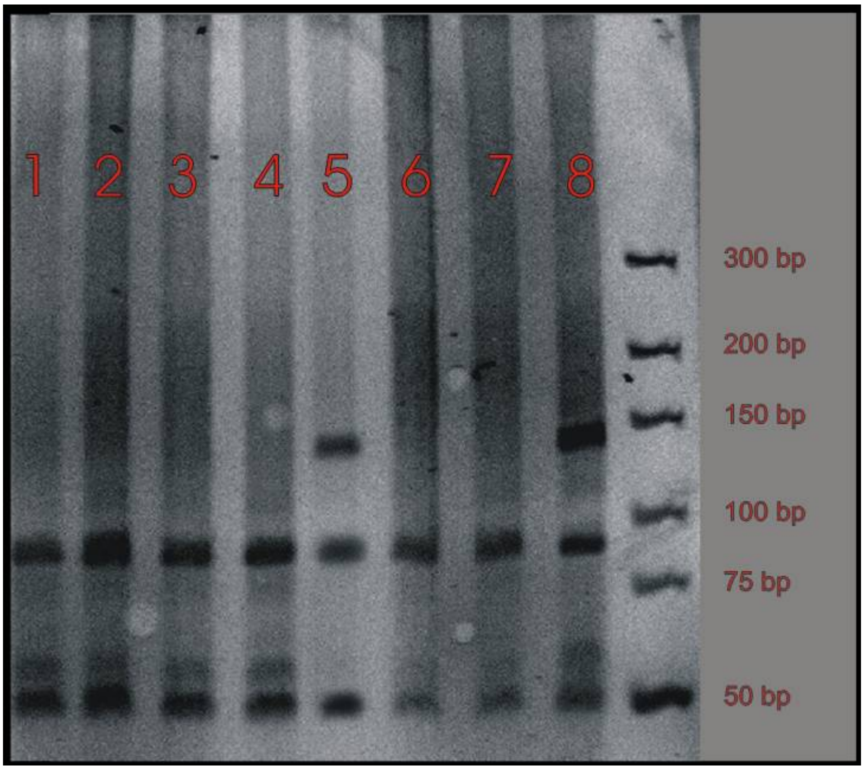

Figure 2. PCR-RFLP at *RYR1* locus. Lanes 1, 2, 3, 4, 6 and 7: Cinta Senese (1843C 18843C); Lane 5: Cinta Senese (1843C 1843T); Lane 8: Pietrain (1843C 1843T).

CONCLUSIONS

Considering the low frequency of the negative allele at *RYR1* locus in Cinta Senese, it seems feasible to eradicate it with any effect on the inbreeding level of the breed. This study confirms the absence of the RN$^-$ allele in the whole breed. Further studies to confirm the obtained results in a larger sample and eventually to plan studies on associations between these DNA markers and meat quality traits in Cinta Senese breed would be desirable.

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SNPs AND MICROSATELLITE MARKERS ANALYSIS FOR GENETIC DIVERSITY STUDY IN ITALIAN PIG BREEDS

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SUMMARY - The use of molecular markers in functional genes as tool to characterize the genetic diversity in livestock populations can provide new opportunities for the identification of putative alleles of economic value. The aim of this study was to investigate of the genetic diversity among eleven pig breeds by typing SNPs in 23 coding genes in five Italian native pig breeds (Calabrese, Casertana, Cinta Senese, Mora Romagnola and Nero Siciliano) and six cosmopolitan breeds (Italian Large White, Italian Landrace, Italian Duroc, Belgian Landrace, Hampshire and Piétrain). A sample of Meishan breed was also included. Allele frequencies, heterozygosity, Fst, Fis, Fit, and Reynolds genetic distances were calculated and Neighbor-Joining tree with bootstrap resampling was drawn. The results obtained for SNPs were compared with those previously produced in PigBioDiv project that analysed DNA samples of some of the breeds of the present study were analysed with microsatellites markers.

Key words: Italian pig breeds, SNP, microsatellite, genetic diversity.

INTRODUCTION

To genetically characterise local breeds molecular markers are an indispensable tool to understand population genetic structure and to trace the presence and the spread of economically important alleles. Till now European local pig breeds have been analysed within the EC funded project PigBioDiv where many pig breeds reared in several European countries, including Italy, were typed with microsatellites (SanCristobal *et al*., 2006a) and AFLPs (SanCristobal *et al*., 2006b). Until now only few researches have been carried out with the aim to analyse the genetic variability using SNPs of genes coding for proteins with known functions. As the Italian pig breeds concerns our research group, we analyzed the genetic relationships among cosmopolitan pig breeds reared in Italy using RFLPs detected by Southern analysis (Davoli *et al*., 1996) and SNPs (Russo *et al*., 2004a). Today in Italy only six local breeds are still present: Casertana, Calabrese, Cinta Senese, Mora Romagnola, Nero Siciliano and Sarda. Additional local ancient pig breeds disappeared some decades ago because they were replaced by selected and more productive ones. In Italy ANAS (National Association of Pig Breeders) is in charge to keep the data recording of pigs of these endangered breeds in the Pig Breeding Registry with the purpose of breed preservation and to apply a management scheme to reduce inbreeding. The protection and valorisation of the endangered Italian local breeds consider their rusticity and hardiness and the high quality of fresh meat and seasoned products, particularly appreciated by the consumers. With the present work we investigated the genetic variability in eleven pig breeds reared in Italy by typing 23 SNPs in coding genes. Moreover, a first comparison of SNPs data with the results of the PigBioDiv project was performed.

MATERIAL AND METHODS

Pigs belonging to cosmopolitan and local breeds reared in Italy were sampled for the present research. Furthermore, some samples of the Chinese Meishan breed were included as outgroup for some genetic analyses. The sampled pigs were chosen trying to exclude full sibs and half sibs. DNA samples were extracted from blood. Some of the samples used for this research were already used in the PigBioDiv project where a panel of 50 microsatellites was analysed (SanCristobal *et al*., 2006a). A detailed list of the utilised samples is reported in Table 1.

Table 1. Minimum and maximum number of animals typed for each breed.

Breed		Range of tested animals per locus
Italian Large White	ITLW	23-73
Italian Landrace	ITLR	17-57
Italian Duroc	ITDU	22-61
Belgian Landrace	BL	19-33
Piétrain	PI	20-50
Hampshire	HA	20-29
Cinta Senese	ITCS	10-15
Casertana	ITCT	14-28
Calabrese	ITCA	8-16
Nero Siciliano	ITNS	20-31
Mora Romagnola	ITMR	11-18
Meishan	MS	5-14

For this research we analysed SNPs detected within 23 loci coding for functional proteins and mapping on 12 different pig chromosomes. The names, symbols and cytogenetic location of all loci and the references describing the chosen SNPs are reported in Table 2.

Table 2. List of the utilised loci.

Symbol	Name of the gene	References	Chromosomal location on pig karyotype
ESR1	Estrogen receptor 1	Short et al. (1997)	1p25-p24
MC4R	Melanocortin 4 receptor	Kim K.S. et al. (2000a,b)	1q22-q27
CTSF	Cathepsin F	Russo et al. (2004b)	2p
LDHA	Lactate dehydrogenase	Fontanesi et al. (2003)	2p17-p14
HUMMLC2B	Myosin regulatory light chain 2	Davoli et al. (2003b)	3p16-p17
CA3	Carbonic anhydrase	Davoli et al. (2006)	4q11-q14
DECR1	2,4-dienoyl CoA reductase 1, mitochondrial	Davoli et al. (2002)	4q15-q16
ATP1A2	Na+, K(+)-ATPase subunit alpha 2	Blazkova et al. (2000)	4q21-q23
RYR1	Ryanodine receptor 1	Fuji et al. (1991), Russo et al. (1993)	6q12
FABP3	Heart fatty acid-binding protein	Gerbens et al. (1997)	6q22-q26/q31-qter
PKM2	Pyruvate kinase, muscle	Fontanesi et al. (2003)	7q12-q23
SLN	Sarcolipin	Fontanesi et al. (2001)	9p24-p21
GAA	Glucosidase, alpha; acid	Fontanesi et al. (2003)	12p13-p11
FASN	Fatty acid synthase	Munoz et al. (2003)	12p15
MYH4	Myosin, heavy polypeptide 4, skeletal muscle	Davoli et al. (2003b)	12q11-q15
ADIPOQ	Adiponectin	Dai et al. (2006), Dall'Olio et al. (2006)	13q36-q41
CSTB	Cystatin B	Russo et al. (2002)	13q46-q49
CTSB	Cathepsin B	Russo et al. (2002)	14q15-q16
MYPN	Myopalladin	Davoli et al. (2003a)	14q25-q29
PRKAB1	Protein kinase, AMP-activated, beta 1 non-catalytic subunit	Fontanesi et al. (2003)	14q22-q24
PRKAG3 (I199V)	AMP-activated protein kinase gamma subunit	Milan et al. (2000), Fontanesi et al. (2003)	15q12
TTN	Titin	Davoli et al. (2003a)	15q23-q26
PGAM2	Phosphoglycerate mutase 2	Fontanesi et al. (2003)	18q13-q21

For each breed, allele frequency, observed and expected heterozygosity (Ho, He), F statistics (Fis, Fst, Fit), were calculated with Genetix 4.05 (Belkhir *et al*. 2002), Arlequin 3.11 (Excoffier *et al*., 2005), and a specific program used to correct He for unequal sample size. Hardy-Weinberg equilibrium was tested for each locus and for each breed using Genepop 3.4 (Raymond & Rousset, 1995). A graphical representation of marker estimated similarity was produced using the method indicated by Eding *et al*. (2002). Reynolds genetic distances (*D*; Reynolds et al., 1983)) were calculated with a program written to correct for unequal sample size. The genetic distances were used to perform Neighbor-Joining clustering with 1,000 bootstrap resamplings with Phylip 3.65 (Felsenstein, 1989). The regression of He vs. distance of population to origin for SNPs (present work) and microsatellites typed in the PigBioDiv project was fitted using the R software (http://cran.r-project.org/).

RESULTS AND DISCUSSION

Allele frequencies in the analysed breeds were calculated using a minimum sample of 17 pigs for the cosmopolitan breeds (ITLW, ITLR, ITDU, BL, PI, HA) and of at least 8 pigs for the Italian endangered breeds taking into account their very low numeric consistency (Table 1). The utilised samples were genotyped for a different number of loci,however each animal was analysed for a minimum of 8 SNPs. The Hardy-Weinberg equilibrium was tested and significant differences for P<0.05 (Table 3) were found for 15 out of 276 test performed. On the whole since the number of significant differences from equilibrium detected was limited (5.4%), all the obtained data were maintained for the subsequent analyses. The observed and expected heterozygosity were calculated for each breed both for SNPs and microsatellites (Table 4).

Table 3. Hardy-Weinberg equilibrium calculated for each breed within locus. Only the results significant for P<0.05 were reported. The P values <0.01 are underlined.

Locus	Breed	P
LDHA	MS	0.038
CA3	PI	<0.001
ATP1A2	ITCA	0.023
RYR1	HA	0.004
FABP3	ITNS	0.020
PKM2	ITNS	0.012
PKM2	ITMR	0.030
FASN	ITCA	0.002
FASN	ITMR	0.015
CTSB	PI	0.050
CTSB	HA	0.022
CTSB	ITCA	0.036
PRKAG3	BL	0.037
PRKAG3	ITCA	0.011
TTN	ITCA	0.046

Table 4. Observed (Ho) and expected (He) heterozygosity calculated for SNPs and microsatellites in each breed.

Breed	SNPs		Microsatellites	
	Ho	He	Ho	He
ITLW	0.35	0.34	0.61	0.61
ITLR	0.31	0.32	0.62	0.62
ITDU	0.27	0.30	0.54	0.55
BL	0.31	0.33	-	-
PI	0.31	0.33	-	-
HA	0.28	0.30	-	-
ITCS	0.25	0.27	0.48	0.47
ITCT	0.32	0.33	0.54	0.59
ITCA	0.28	0.29	0.51	0.47
ITNS	0.29	0.29	0.64	0.67
ITMR	0.20	0.19	-	-
MS	0.14	0.14	0.58	0.59

SNPs average He ranged from 0.14 in MS to 0.34 in ITLW. For microsatellites values of He of 0.47 or higher were found: these values are expected because microsatellites presented several alleles in comparison with SNPs that are usually biallelic. It is noteworthy to observe the heterozigosity of the ITNS local breed that is notable for SNPs and very high for microsatellites. A possible origin of this high variability could be dueto the introduction of some Italian and English breeds during the 18th Century to be crossed to Sicilian pig populations in order to improve the productive traits of the pigs reared in Sicily (Chiofalo and Liotta, 2003). The F-statistics and their P-values were calculated for all loci genotyped with SNPs (Table 5).

Table 5. F-statistics among breeds over 23 loci.

Locus	Fis	Significance level	Fst	Significance level	Fit	Significance level
ESR	-0.192	NS	0.489	***	0.391	***
MC4R	-0.008	NS	0.297	***	0.292	***
CTSF	-0.051	NS	0.137	***	0.093	NS
LDHA	0.045	NS	0.028	*	0.072	NS
HUMMLC2B	-0.048	NS	0.299	***	0.266	***
CA3	0.217	***	0.210	***	0.382	***
DECR1	-0.050	NS	0.087	***	0.042	NS
ATP1A2	0.038	NS	0.192	***	0.223	***
RYR1	0.341	**	0.802	***	0.870	***
FABP3	0.074	NS	0.316	***	0.366	***
PKM2	-0.005	NS	0.061	***	0.056	NS
SLN	0.112	NS	0.078	***	0.181	**
GAA	-0.051	NS	0.211	***	0.171	***
FASN	-0.065	NS	0.153	***	0.098	NS
MYH4	0.001	NS	0.088	***	0.089	NS
ADIPOQ	0.195	NS	0.043	*	0.229	*
CSTB	0.250	***	0.139	***	0.354	***
CTSB	0.137	**	0.155	***	0.271	***
MYPN	0.030	NS	0.260	***	0.282	***
PRKAB1	-0.016	NS	0.414	***	0.405	***
PRKAG3	0.120	*	0.116	***	0.223	***
TTN	0.050	NS	0.201	***	0.241	***
PGAM2	-0.019	NS	0.460	***	0.450	***

* = P<0.05; ** = P<0.01; *** = P<0.001; NS = not significant.

Table 5 shows that the differences among populations (Fst) are significantly different from zero for all loci whereas those within each population (Fis) are significantly different from zero only for *CA3, CSTB, CTSB, PRKAG3* and *RYR1*. Fst statistic showed very high scores with an average over all loci of 0.24. In particular eight loci exceed the value of 0.25 and among them five exceed 0.30, supporting a high level of genetic differentiation among breeds for the considered loci. These data are in agreement with those obtained in the PigBioDiv project using microsatellites where the average value of Fst was 0.21.

Figure 1 shows a graphical representation of the average homozygosity within breed on the diagonal: the lowest levels are indicated by white and yellow squares while the highest levels are represented by red squares. From the figure, it is clearly visible that MS and ITMR are the most inbred breeds. Furthermore, the average similarities between pairs of breeds drawn with the same scale of colours were represented above and below the diagonal. The most similar breeds in this comparison were ITCS and HA while MS compared to ITBL, ITCA and ITLW were the most different ones.

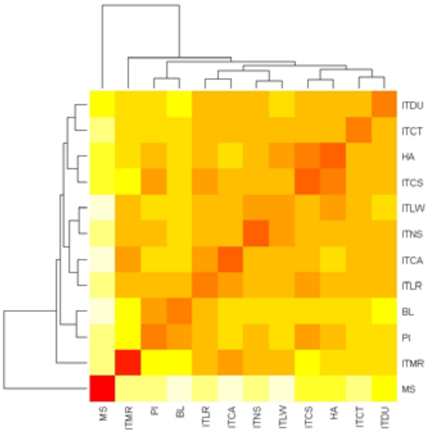


Figure 1. Colour representation of the average similarity matrix (row and columns ordered with a hierarchical clustering).

The calculated Reynolds genetic distances showed, as expected, the extreme genetic difference between the Chinese breed Meishan and the European ones (Figure 2). Among the latter Mora Romagnola is the most diverse and this may probably partially be due to the high level of homozygosity (Table 2 and Figure 1) as consequence of a severe bottleneck that occurred recently in this breed (Zambonelli and Bigi, 2006). The highest values of the bootstrap were obtained for the branches of the dendrogram including ITLW and ITNS (73%), HA and ITCS (63%), PI and BL (62%).

Figure 2. Neighbor-Joining tree based on modified D. Numbers indicate the occurrence of bootstrap resamplings >60%.

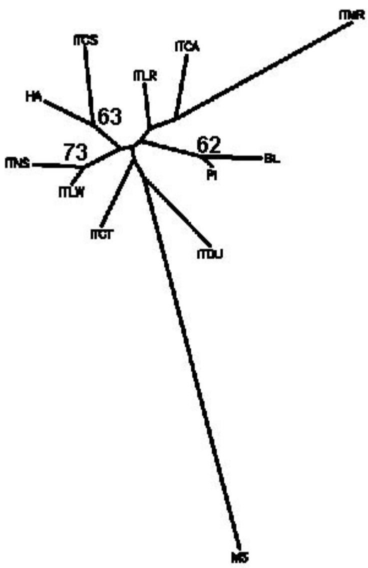
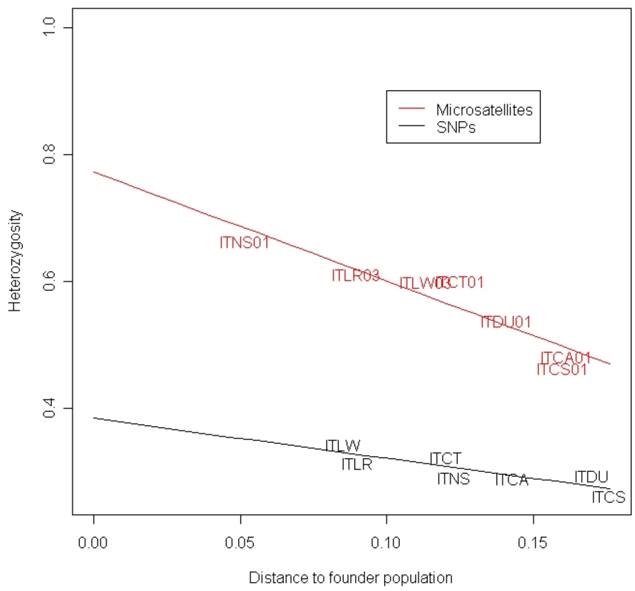


Figure 3. Regression of expected heterozygosity vs. distance to founder population calculated for microsatellites (upper line) and SNPs (lower line).



A preliminary SNP and microsatellite based regression of He vs. distance of each population to the origin was obtained (Figure 3). This analysis indicates that the founder He is 0.38 for SNPs and 0.77 for microsatellites; both intercept values are highly significant: the latter is of the same order as for the whole PigBioDiv population set including all European breed analysed. From Figure 3 it is possible to observe that the ranks of breeds among the two datasets seem stable, except for ITNS, showing the highest heterozygosity with microsatellites, but medium with SNPs. The concordance of the trend obtained with the two types of markers and with the graph obtained with the whole PigBioDiv dataset could indicate that, in agreement with the hypothesis reported by SanCristobal et al. (2006a), genetic drift was the main evolutionary force that differentiates European pig breeds.

CONCLUSIONS

This work describes the first results of genetic characterization of native Italian pig breeds using 23 SNPs. The obtained data indicate that the pig breeds reared in Italy show a good level of genetic differentiation. Furthermore, the comparison of the parameters of genetic variability calculated with SNPs and those produced within the PigBioDiv project using microsatellites showed concordance of results. It is relevant to note the variation of Fst across loci and the quite high values for this parameter indicating that there are important differences among breeds that should be further investigated to validate them and to check if this evidence might be due to a certain level of signature of selection for some breeds and some loci considered in this research.

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GENETIC ANALYSIS OF THE *KIT* AND *MC1R* COAT COLOUR AFFECTING GENES IN NERO SICILIANO PIG BREED AND PERSPECTIVES ON THE USE OF MUTATIONS AT THESE TWO LOCI FOR BREED TRACEABILITY

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SUMMARY - In this study we analysed two coat colour affecting genes, *KIT* and melanocortin 1 receptor (*MC1R*), with the aim to i) characterize the Nero Siciliano (Sicilian Black) pig breed at these two loci, ii) identify mutations associated with the presence of white coat colour patterns in some animals of this breed and iii) identify DNA markers useful for breed traceability of meat obtained from Nero Siciliano pigs. Two samples were considered in this study: 104 pigs collected from 13 different farms, without recorded information on coat colour; ii) 60 pigs sampled from 2 farms with recorded coat colour data. Four alleles (E^{D2}, E^{P}, E^{D} and *e*) have been identified at the *MC1R* locus, thus markers at this gene cannot be used for Nero Siciliano product traceability. All analysed animals did not carry the exon/intron 17 splice site mutation of the *KIT* gene. Thus, the meat of Nero Siciliano pigs could be distinguished from meat of white pigs that have this mutation. As only 4 out of 12 pigs with white patches were the only carriers of the I^{p} allele at the *KIT* locus, white coat colour portions in this breed may be also determined by other mutation(s) of the *KIT* gene or influenced by different genes.

Key words: *MC1R* gene, *KIT* gene, Nero Siciliano pig breed, molecular breed traceability

INTRODUCTION

Nero Siciliano (Sicilian Black) is an autochthonous pig breed reared in the internal areas of Sicily island (Italy) mainly in the Nebrodi mountains. Its breed heardbook was establish in 2001 and, at present, the animals of this breed are reared in more than 100 farms that account, on the whole, about 700 gilts (ANAS, 2008). The animals are usually completely black with a dorsal stripe but a few present white portions mainly in the face or in the fore legs.

Due to the recent increase of the market for local and typical products, the Nero Siciliano breed has been rediscovered by farmers who have established a consortium with the aim to protect, valorise and characterise the products obtained from this pig breed. As the meat obtained from these pigs is sold at a higher price than that of commercial pigs, a request to label the fresh Nero Siciliano meat with the Protected Denomination of Origin (PDO) was issued in 2005 (D'Alessandro et. al. 2007). The higher price of its meat products needs to establish traceability and authentication systems that may guarantee the pig farmers, the production chain as well the consumers from faked products. Recently, to verify the information reported on the product labels, DNA systems based on the analysis of mutations in genes affecting breed specific phenotypic traits, like for example, the coat colour (Maudet and Taberlet, 2002; Russo and Fontanesi, 2004; Russo et al., 2007), have been applied.

In pigs, two main loci (*Extension and Dominant White*) have been shown to affect coat colour in several breeds. The *Extension* (*E*) locus codes for the melanocyte stimulating hormone receptor (MSHR) or melanocortin 1 receptor (MC1R) that is expressed in melanocytes (Robbins et al., 1993). MC1R is a member of the superfamily of G-protein-coupled receptors consisting of seven transmembrane domains whose action on eumelanin synthesis is mediated, upon binding of the α-MSH and ACTH peptides, through the activation of adenylyl cyclase to elevate cAMP levels in melanocytes, that in turn affect tyrosynase activity (Mountjoy *et al.*, 1992). Classical genetic studies have indicated that dominant alleles at the *Extension* locus are associated with black coat colour while recessive alleles at this locus produce red/yellow coat colours (Searle, 1968). Several mutations of the *MC1R* gene have been described in mouse (Robbins et al., 1993), human (Valverde et al., 1995) and in different farm mammals, like cattle (Klungland et al., 1995), sheep (Våge et al., 1999), horse (Marklund et al., 1996) and rabbit (Fontanesi et al., 2006). Some of them cause a constitutive activation of the MC1R protein dependent signaling pathway inducing eumelanin synthesis (black coat colour) while others cause a loss of function of the coded protein and induce phaeomelanin production (red/yellow coat colour). In pigs several alleles at the *Extension/MC1R* locus have been identified and characterized at the molecular level (Kijas *et al*., 1998; 2001): alleles MC1R*1 and MC1R*5 for the wild type colour (allele E^+); alleles MC1R*2, MC1R*7, and MC1R*3 for the dominant black (alleles E^{D1} and E^{D2}); allele MC1R*6 for red or white with black spots (allele E^p); and allele MC1R*4 for the recessive red (allele *e*) (reviewed in Andersson 2003).

The Dominant White locus codes for the *KIT* gene for which several mutations have been identified. The Dominant White alleles (*I*) are caused by the presence of copy number variation (duplication of the whole gene) and a splice site mutation (G>A) in intron 17 in one copy of the duplicated gene that eliminates a splicing site (Marklund et al., 1998; Pielberg et al., 2002). In some alleles (I^2 and I^3), more than two copies of the *KIT* gene (carrying or not carrying the splice site mutation) are present. Other alleles have been identified at this locus: I^p that is caused by a duplication of the *KIT* gene without presence of the splice site mutation and the I^{Be} that should cause the Belted phenotype but for which the causative mutation has not been identified yet (Giuffra et al., 1999).

The aim of this study was to investigate the *MC1R* and *KIT* loci in Nero Siciliano pigs in order to evaluate if DNA markers at these two coat colour affecting genes can be used for breed traceability and authentication of meat products obtained from this breed.

MATERIAL AND METHODS

DNA was isolated from blood samples collected from 104 pigs from 13 different farms (Russo et. al 2004), without recorded information on coat colour, and from 60 pigs sampled from 2 farms, with recorded coat colour.

The intronless porcine *MC1R* gene was analysed amplifying three PCR fragments containing the polymorphic sites that make it possible to distinguish the E^+, E^{D1}, E^{D2}, E^P and *e* alleles (Kijas et al., 1998, 2001; Russo et al., 2004b). Analysis of the first PCR fragment can distinguish the E^P allele (determined by a 2 bp insertion that causes a frame shift at codon 23) from the other alleles (Kijas *et al*., 2001). The amplified products of this portion of the gene were revealed by capillary electrophoresis on an ABI3100 Avant (Applied Biosystems) sequencer. PCR-RFLP analyses were carried out using the other two amplified fragments of 196 and 154 bp, respectively (Russo et al., 2004b). The 196 bp fragments were digested with *Bsp*HI that elucidates the mutation at codon 124 and the 154 bp fragments were digested with *Mvn*I and *Hha*I that detect the two mutations at codon 243 (Kijas et al., 1998; Russo et al., 2004b). The obtained fragments were resolved by means of polyacrylamide gel electrophoresis and visualized with ethidium bromide.

For the *KIT* gene, two PCR products were amplified and analysed according to Marklund et al. (1998) and Giuffra et al. (2002), respectively: 1) a 175 bp region containing the splice site mutation that was digested with *Nla*III; 2) a 152 bp product spanning the 3'-5' breakpoint as a diagnostic test for the *KIT* duplication region. As the duplication test rely on the presence or absence of the targeted PCR region it is possible that failure of the PCR might erroneously attribute a genotype to the analysed animals. In order to avoid this problem, we designed a duplex PCR protocol to amplify in the same tube a fragment of the single copy gene *ESR* (as an internal PCR control) and the 152 bp kit duplication test. PCR products and digested fragments of the *KIT* gene were analysed as described above.

RESULTS AND DISCUSSION

Figure 1 reports the four assays designed to analyse the porcine *MC1R* gene. The combination of these four assays made it possible to assign the genotypes at this locus. Considering the two groups of analysed pigs, four alleles (E^{D1}, E^{D2}, E^{P} and *e*) have been identified at the *MC1R* locus. In the first group of pigs (104 animals without recorded coat colour information) the frequency of the *MC1R* alleles was the following: 0.187, 0.673, 0.106 and 0.034 for alleles E^{D1}, E^{D2}, E^{P} and *e*, respectively. Six different genotypes were observed among which the most frequent was E^{D2}/E^{D2} (0.38). In the second group (with recorded coat colour information) alleles E^{D1} and *e* were not observed, maybe due to the sampling that was conducted in a smaller number of farms. Allele frequencies of this second group were 0.784 and 0.216 for alleles E^{D2} and E^{P}, respectively. Combining the two groups, allele frequencies were 0.119, 0.713, 0.146 and 0.021 for alleles E^{D1}, E^{D2}, E^{P} and *e*, respectively. These data indicated that the *MC1R* gene is not useful for breed traceability of Nero Siciliano products because this breed is not fixed or almost fixed for any alleles making it impossible to distinguish, or at least exclude, based only on *MC1R* polymorphisms, other breeds that carry the same alleles (Table 1).

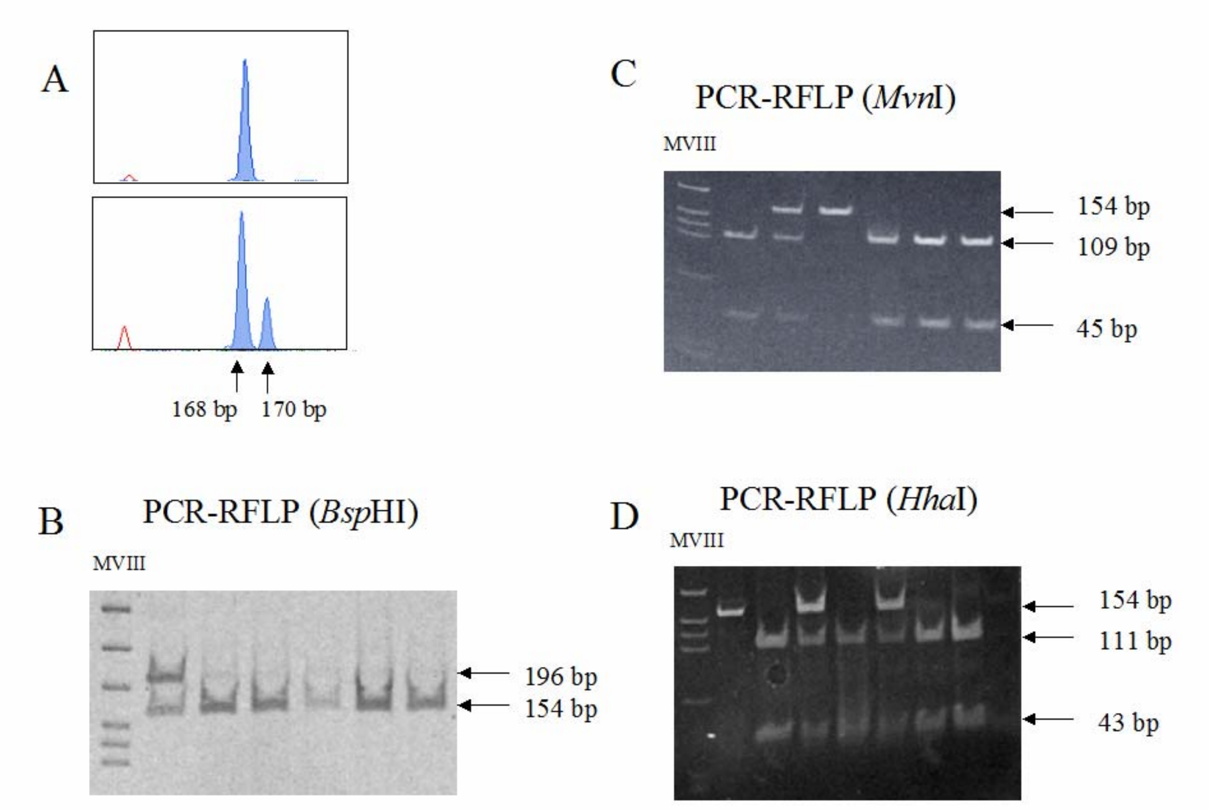

Figure 1. The four assays designed to analyse the porcine *MC1R* gene. A: capillary sequencer analysis of the 2 bp insertion. B, C and D. PCR-RFLP tests. The fragment of 42 bp resulted from the digestion with *Bsp*HI of the 196 bp product is not shown in the gel (subset B).

Figure 2 shows the two assays designed to investigate the *KIT* gene. All analysed animals were negative for the intron 17 splice site mutation of the *KIT* gene. That means all animals carried G instead of A at this polymorphic site (Marklund et al., 1998). Thus, the meat of Nero Siciliano pigs can be distinguished from meat of white pigs that are positive for this polymorphic site (Table 1).

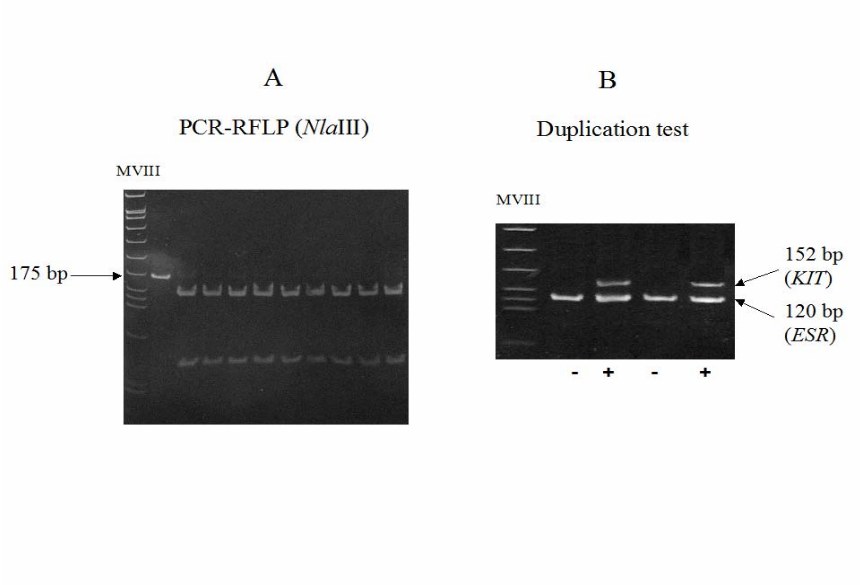

Figure 2. A) PCR-RFLP analysis at the *KIT* locus to analyse the splice site mutation. B) Duplication test for the *KIT* gene.

Of the first group of pigs, only 5 were positive at the duplication test of the *KIT* gene. In the second group of pigs, in which coat colour was recorded, only 4 out of 12 pigs with white patches resulted positive at the duplication test. Animals positive at the duplication test carried the I^p allele. Considering that all animals that were positive at the duplication test might be heterozygous for this allele (due to the low frequency), combining the two groups of pigs the frequency of the I^p allele in the Nero Siciliano population was 0.027. Due to its low frequency, it could be possible to eliminate this allele from the breed if this could be useful to improve the exclusion probability of the *KIT* mutations in an authentication test for Nero Siciliano meat.

Summing the results of the two investigated loci (*MC1R* and *KIT*) and comparing the results obtained for the Nero Siciliano breed with the data reported in the literature (Marklund et al., 1998; Giuffra et al., 1999; Giuffra et al., 2002) and from our unpublished results for other breeds (Table 1) it is possible to evaluate that the use of the *KIT* locus could be useful, even if in not all cases, to exclude the fact that meat of white animals are labelled as obtained from Nero Siciliano pigs.

Table 1. Comparison between Nero Siciliano alleles and those of other breeds for the *MC1R* and *KIT* loci. Asterisk indicates that the labelled allele is at low frequency.

Breeds	*MC1R* alleles	KIT		
		Splice site mutation	Duplication	Alleles
Large White	E^P	A/G*	+/-*	I^1, I^2, I^3, I^p*
Landrace	E^P	A/G*	+/-*	I^1, I^2, I^3, I^p*
Pietrain	E^P	G	-/+	*i*, I^p
Duroc	*e*	G	-	*i*
Wild boar	E^+	G	-	*i*
Meishan	E^{D1}	G	-	*i*
Hampshire	E^{D2}	G	-	*i*
Nero Siciliano	E^{D2}, E^{D1}, E^P, *e**	G	-/+*	*i*, I^p*

CONCLUSIONS

The presence of more alleles at the *MC1R* gene in the Nero Siciliano breed makes this locus not useful for breed traceability of its meat products. Thus, the meat of Nero Siciliano pigs can be distinguished from meat of white pigs that are positive for this polymorphic site. In the group with recorded coat colour, as only 4 out of 12 pigs with white patches were the only carriers of the I^p allele at the *KIT* locus, white coat colour portions in this breed might be also determined by other mutation(s) of the *KIT* gene or affected by different genes.

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VARIATION OF MICRONUCLEI FREQUENCY IN LYMPHOCYTES OF ANCIENT 'CASERTANA' AUTOCHTHONOUS PIG GENETIC TYPE (AAGT) IN RELATION TO PHOTOPERIOD. PRELIMINARY RESULTS

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SUMMARY - The living organisms are furnished of systems that allow to modify their behaviour in relation to the variations of cosmic, physical, chemical, biochemical factors, in relation to the 'bioterritory' (agri-ecosystem) in which they are inserted; some of these factors are the rotation and revolution movement of the Planet Earth. One of the more studied biological rhythms is the 'circadian' one, regulated by daily light/dark hours ratio and able to influence the daily biochemical, physiological and behavioural activities of both animals and plants. Up today, for the most of animal species, little knowledge about molecular bases of circadian rhythm exists. Micronuclei Test (MN) was employed to notice the possible incidence of the photoperiod on the genome stability. The technique employed was that of Matassino *et al.* (1994), opportunely modified on lymphocytes of peripheral venous blood. Research was carried out on 37 males and 33 females of 'Casertana' AAGT. In the limits of the observation field, in function of the photoperiod, the binucleated cells percentage with 1 and 2 MNs: (i) does not change in relation to the age, in both sexes whereas is significantly variable($P < 0.031$), for the males, only for binucleated cells with 2 MNs; (ii) evidences that the best fit function is that of IV order ($P<0.0001$ in the female and $P<0.05$ in the male, for the binucleated cells with 1 MN; $P<0.01$ in the female and 20 % significance in the male, for the binucleated cells with 2 MNs) with the maximum values in correspondence of the two equinoxes (spring and autumn) and minimum values in coincidence with the two solstices (winter and summer); and (iii) is significantly variable in the sexes for the binucleated cells with 1 MN (male/female ratio=11) whereas it is nearly unchanged in the cells with 2 MNs (male/female ratio =1.4).

Key words: photoperiod, micronucleus, pig, 'Casertana '.

INTRODUCTION

Genome instability, both natural (aging) and induced by physical, chemical and/or environmental agents may cause chromosomal structural aberrations with a consequent loss of some genome portions. A part of these aberrations give rise to chromosomal fragments lacking of kinetochores or centromeres, called 'acentric fragments' (AF). When the cell divides, some of these fragments are excluded from the nucleus of the daughter cells producing small nuclei inside the cytoplasm, called 'micronuclei' (MN). Depending on the origin of such fragments, both or one of the daughter cells will suffer a genetic loss. The determination of the MNs frequency constitutes a valid, simple and fast test, to reveal a DNA damage caused by physical and/or chemical and/or environmental agents. In comparison to the other tests commonly employed to estimate the genome stability [SCE test (Sister Chromatid Exchange) and Comet test], the MNs test is a simple technique and furnishes information about previous DNA damage too.

The photoperiod is a biologically important physical factor influencing, among the other things, the biological activity of a cell.

In *Drosophila Melanogaster*, the most investigated organism in relation to the photoperiod, several polypeptide/s coding DNA segments ('*genes*') involved in the circadian rhythms have been identified, amongst them *timeless* (*tim*) whose allele '*ls-tim'*, is responsible for synthesis of the two protein isoforms, *L-TIM* and *S-TIM*, arising from alternative splicing (Rothenfluh A. *et al*., 2000; Hardin P.E., 2005). Physical and/or chemical and/or environmental agents may induce genome instability in such DNA segment, so that they can favour mutations, rearrangements, deletions with consequent effects on the expression regulation of the above segment.

The term 'chronogenetics' was introduced by Gedda and Brenci (Gedda L. *et al.,* 1973) to point out the 'original hereditary time' being hereditarily transmitted and subjected to the genetic variability; therefore, it is a primitively 'biological' or 'endogenous' time, as effect of the 'life' phenomenon. Therefore, 'chronogenetics', involving endogenous temporal greatness, have to be distinguished by 'chronobiology' which, instead, involving relationships between 'physical' and 'biological' time, is expressed as 'reactive time'.

The importance of 'chronogenetics' is linked to temporal (both qualitative and quantitative) behaviour of each polypeptide/s coding DNA segment. Therefore, such a segment possesses a fourth dimension that characterizes the duration of its information; consequently, also the temporal variability has to be taken into account in the variability of formal genetics.

The external rhythmic fluctuations are epiphenomena that the DNA segment 'receives', 'transmits' and 'consumes'. Therefore, the 'exogenous' time is a rhythmic time of solicitation of vital phenomena sustained by a 'primitively biological time'. This solicitation concerns only the functional mechanisms of genome. The 'astral' time would activate an 'operative' DNA segment ; this latter would activate a polypeptide coding DNA segment and maintain it active up to the moment in which the exogenous stimulation returns below the operative threshold of the 'regulative' DNA segment.

An *ante litteram* demonstration of 'chonogenetics', identifiable with 'florae clock' was given by Linneo C. in 1735, before the discovery of Mendel's laws (1865).

In conclusion, the 'biological individuality' may not identify itself only with the 'quality' and 'quantity' of the information of a polypeptide or non polypeptide coding DNA segment, but it must take into account its 'temporality', which is function of the so-called DNA segment 'stability' defined as 'ergon'. According to Gedda and Brenci (1973), the main factors of stability should be identified with: (i) 'synonymy' or 'molecular stability; (ii) 'redundance' or 'stability due to repetitive information'; and (iii) 'repair or 'stability due to DNA repair'.

DNA segment must be studied and known in its informative effects concerning different moments, strongly connected to each other, hence at level of quality, stability ('ergon') and duration ('chronon').

Fundamentally, the 'ergon-chronon' relationship represents an irreplaceable system for the function of any biological entity inserted in a given microenvironment; this 'external' microenvironment, in addition to the 'inner' one, would influence the 'temporal dimension' of the 'physiology' and 'function' of DNA segment, such as the output speed of information. Similarly to phylogenesis of inherited characters, there is a 'chronon' phylogenesis .

The aforesaid two variables ('ergon' and 'chronon') are interdependent in the sense that to a given stability of a DNA segment in a given microenvironment corresponds a 'hope of life 'of the information ('chronon'). This complex biological phenomenon stresses the importance of the epigenetics, hence of the 'epigenome'.

During the evolution, the various DNA segments have incorporated the time (or they may incorporate a chronon), so that at both individual and population level repetitive demonstrations temporally closed, defined 'family isochronism' by Bettini (1977), occur within a 'family' (ethnic group sharing a given kinship degree); therefore, the 'hereditary responsible unit' has in itself a 'temporal measure of information' in terms of both its 'activity' and its 'extinction' (Matassino et al., 2006; Matassino et al., 2007).

Chronogenetics is expressed at different levels of biotic system organization: submolecular, molecular, cellular, tissue, organic, organism, biocenotic and ecosystem levels.

The 'rhythm' or better the 'biorhytm' is detectable in 'biochemical', 'enzymatic' , 'physiological', 'electrochemical', 'behavioural'(emotionalism, ecc.), 'intellectual' processes of any living being. The biorythm is a 'biological oscillator' and it may be defined as a periodic variation of a given event. The rhythms may be divided into: (i) autonomous rhythms, if they act as physical oscillators independently on the presence/absence of external strengths, that is independently on the 'periodism of field'; (ii) rhythms of spontaneous activity, if the synchronization, the external (environmental) or inner (hormone) factor varying in accordance with a periodic course, requires the intervention of 'biological clocks'; and (iii) rhythms triggered by physical oscillators or rhythms as answer to environmental cycles, if they behave as physical oscillators synchronized to periodic field variables. Taxonomically, biorythm is classified into: (i) ultradian (t < 20 hours); (ii) circadian (20 hours< t < 28 hours); (iii) dian (23.8 hours< t<24.2 hours); (iv) infradian (t >28 hours); (v) seven days period (t= 7± 3 d); (vi) fourteen days period (t= 14 ± 3 d); (vii) twenty days period (t= 21 ± 3 d); (viii) thirty days period (t= 30 ± 5 d); and (ix) circannual (t= 1 year ± 2 months).

The rythmics of the biological phenomena is influenced by factors both inner '(endogenous periodicity) and external '(synchronizer); therefore, the rhythmic variations are regulated and coordinated by 'biological clocks' (hormonal release, sleep-control, ecc.), but, contemporarily they are influenced by epigenetic factors. In other words, the inner biological clock is able to foresee the regular changes of the course of the external synchronizers. Any biorythm is conditioned in its course by a 'primary' synchronizer. Any living being is characterized by particular structures that allow to perceive the variation of a 'primary synchronizer' and to reconcile its 'biorythm' with that of the microenvironment in which it lives. The elimination or deep reduction of the effect of an 'external synchronizer' causes a deep variation of biorythms of the organism or population to reach a biorythm to 'raced free'. The most evident and investigated biological rhythm is the circadian one (Matassino, 1988).

With the exception of the *Archea*, most of *Eubacteria*, amongst which *Escherichia Coli*, and some low Eukaryotes such as *Saccaromyces cerevisiae*, all the organisms show circadian rhythms. In the Eukaryotes, environmental regulation mechanisms are important in modulating the expression of polypetide/s coding DNA segments. Bernabucci *et al.* (2006) evidence that the exposure to different photoperiods may modify expression of DNA segment coding for 'leptin' (hormone expressed in adipose and muscle tissue, placenta and mammary gland of various animal species playing a critical role in the regulation of body weight, hematopoiesis, angiogenesis and immune as well as anti-inflammatory answer) and its receptors. Particularly, exposing animals to a 'long day '(18/6 light/dark hours) determined a significant increase of transcriptional activity of leptin and its receptors DNA segments, in comparison with 'neutral day' (12/12 light /dark hours), while the exposure to a 'short day' (6/18 light /dark hours) caused a decrease of this activity in comparison to the 'neutral' condition (Bernabucci *et al.,* 2006).

Within farm species, it seems that the periodic course of some physical-biological and biopoietic characteristics are in relation to photoperiod.

Research carried out on Gentile di Puglia sheep, showed that the photoperiod is able to influence the value of some ematochemical parameters during the year, as well as the activity of some enzymes (Matassino *et al.,* 1975; Rubino *et al*., 1975; Matassino *et al.,* 1982). Indeed in sheep, the 'circannual' course of mean value for total proteins, albumins, □-globulins, serum iron and emocrome was a function of the physical oscillator 'photoperiod'; such course tended to extend to any 'physiological' *status* (pregnancy under induced *oestrus*, pregnancy under physiological *oestrus* and 'not pregnancy'), suggesting that photoperiod, as cosmic factor, is so determinant to erase the effect of 'physiological' *status*. In analogous way, concerning the activity of some enzymes, research evidenced that photoperiod may influence their activity, particularly: (i) protein metabolism and elimination of aminic nitrogen [glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT)]; (ii) production of energetic substrates (creatine- phosphochinase, CPK); (iii) catabolism of many phosphorous compounds (alkaline phosphatase , ALP); and (iv) glycolysis [lactic deidrogenase (LDH) and its isoenzymes (LDH1,LDH2,LDH3,LDH4,LDH5). Also concerning enzymatic activity, the physiological state of the ewe seems to have no significant influence on the *circannual course* of enzymes and isoenzymes investigated. The rhythmic course has put in evidence that the subjects having higher activity of a given enzyme, tend to have a significant higher significantly for all the other enzymes investigated; this assumes notable importance in terms of 'constructivism ability' of the 'single' (Matassino *et al.,* 1975;Rubino *et al*., 1975; Matassino *et al.,* 1982).

It has been shown that in cows, under latitude of the Italian peninsula, on hundreds of thousands of lactations, the mean daily milk yield production at population level and under ‘demographic homeostasis’ condition, tends to vary throughout year reaching the maximum value at ‘end of winter-beginning of spring’ and the minimum value at ‘end of summer-beginning of autumn’ according to a harmonic function. Therefore, it has been hypothesized how such ‘biopoiesis’ would behave according to a ‘biological oscillator’ triggered by a ‘physical oscillator’ (the ‘photoperiod’) (Bettini *et al*., 1969; Matassino *et al.,* 1975; Matassino *et al.,* 1982) with an anticipation of three months of the solstices and the equinoxes.

If in literature many studies exist about DNA segments involved in the regulation of circadian cycle, little research exists about the possible relationship between photoperiod and genome stability despite the undisputed existence of genome -environment interaction. A biological entity is endowed with an adaptative dynamics that allows her to answer to the various environmental challenges that induce this entity to develop its ‘ability to the constructivism’ (fitness) (Matassino *et al .,* 1989; Matassino *et al.,* 1992). To improve this ‘constructivism ability’ each complex system is characterized by ‘self-organization’ or ‘self-assembly’, that is a dowry (or prerogative) of a biological system to locally develop structures orderly ‘from chaotic situations ‘(Matassino *et al.,* 2007). Klir (1991) defined a ‘self-organizing’ system as: ‘A system tending to improve dinamically its own capabilities through a better organization of its elements to achieve the goal’. In the living system ‘self-organization’ confers a further dimension which match the overall complexity with the aim to improve ‘constructivism capability (fitness)’. In our paper the possible relationship between photoperiod and genome stability was investigated through the employment of Micronuclei test. Particularly the possible effect of the photoperiod on genome stability was tested on pig subjects belonging to ‘Casertana’ Ancient Autochthonous Genetic Type (TGAA).

MATERIAL AND METHODS

Micronuclei test was carried out on peripheral blood of 70 (37 entire males and 33 females) ‘Casertana’ AAGT pigs, reared at the experimental Farm of the ConSDABI-NFP.I-FAO in Circello (BN - Italy). The technique used was suggested by Matassino *et al.* (1994) for the bovine lymphocytes, appropriately modified (Matassino *et al*., 2006).

The cell cultures were prepared using 8 ml of RPMI 1640 (Gibco), lymphocyte ring separated from 8 ml of whole blood, 15% of inactivated Foetal Bovine Serum (FBS), 10 ml/ml of L-glutammine and 10 ml/ml of Pokeweed. After growing for 44 hours, cytochalasin-B was added to a 6 g/ml final concentration (Scarfì *et al*., 1993). After 72 hours of growth in thermostat, the cellular suspension was treated with buffer to lyse eritrocytes. After washing in RPMI 1640 (Gibco) enriched with 2% FBS, the cellular suspension was submitted to hypotonic treatment for 15 minutes. The binucleated cells with and without MN were observed under fluorescence microscope Leica with 20x and 100x enlargement. In this paper binucleated cells with 1 MN and 2 MNs were taken into account; the age and photoperiod were considered as fixed factors for mathematical-statistical elaboration; all the percentage values less than 30% and higher 70% were transformed into arcsen.

RESULTS AND DISCUSSION

Within the limits of the observation field: (i) the percentage of binucleated cells with 1 MN ranged from a minimum of 1.01% to a maximum of 2.17% for the females and from a minimum of 11.72% to a maximum of 18.44% for the males; male/female ratio is, on average, equal to 11 throughout the year; monthly this male superiority ranges from a maximum value of 17.4 in July to a minimum value of around 8, in September; and (ii) the percentage of binucleated cells with 2 MNs resulted to vary from a minimum of 0.21% to a maximum of 2.50%, for females, and from a minimum of 0.24% to a maximum of 4.98% for males; male/female ratio is, on average, equal to 1.4 throughout the year.

The photoperiod is significant in female ($P<0.010$) and poorly significant in male ($P<0.09$). In relation to the photoperiod, for both sexes, the binucleated cells percentage with 1 MN varied according to a IV order function with 2 minimum values in June and December (summer and winter solstices) and 2 maximum values in correspondence of March and October (equinoxes nearly to spring and autumn equinoxes) (Fig. 1). The function, is significant in females ($P< 0.0001$) as well as in males ($P<0.05$). The relation between photoperiod and spontaneous MNs percentage might be explained on the basis of what reported by Niekamp *et al*. (2006) in a study about the influence of photoperiod on the immune state of pregnant sows and their offspring (from 7 to 21 days of age); these Authors showed that lymphocyte percentage tends to decrease reaching the minimum value at 16 hours of light. Therefore, the minimum value of lymphocyte percentage with MNs detected in this paper in correspondence of summer solstice might be compatible with decrease of lymphocyte percentage occurred in the same period.

In relation to the photoperiod, the percentage of binucleated cells with 2 MNs tends to vary according to a model (fig. 2) not different from that showed for the binucleated cells percentage with 2 MNs. The function results highly significant (P< 0.01) for the female and significant at 20% for the male. Nawdays, from literature no study exists about the effect of photoperiod on genome stability estimated with MN test.

CONCLUSIONS

The photoperiod might be a significant physical factor influencing the course of genome stability estimated with MicronucleusTest in both males and females of Casertana AAGT pig. The reaction to endogenous factors, such as age, is variable in function of the sex: the female shows a higher genome stability, statistically significant in comparison with male; this behaviour could be the result of the greater *'ability to constructivism'* (fitness) of female to reproduce; this superiority is probably due to a higher effectiveness of the complex *'self-organization'* of female cell. Furthermore, these studies should be regarded the comparison among autochthonous genetic types and cosmopolite breeds, for a better knowledge of the level of *'ability to constructivism'* (fitness) of an ethnic group. The fitness of a genetic type should be a parameter to take into account, useful, for genetic improvement programmes.

Fig. 1. Course of the percentage of binucleate cells with 1 in operation MN of the photoperiod (month). I compare male/female

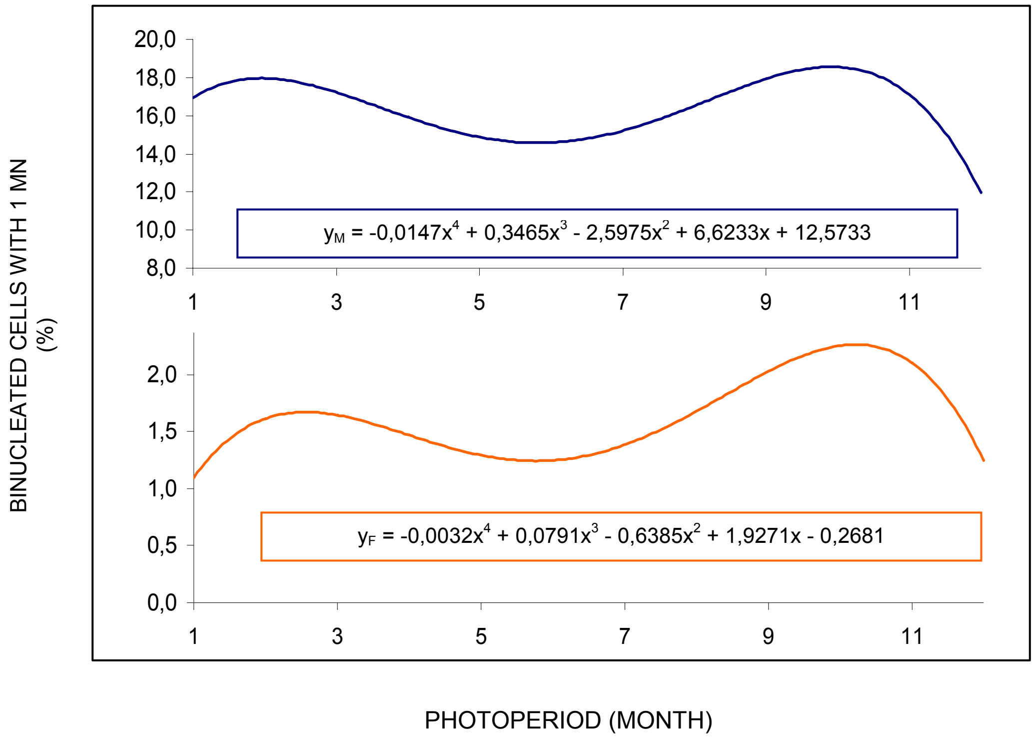
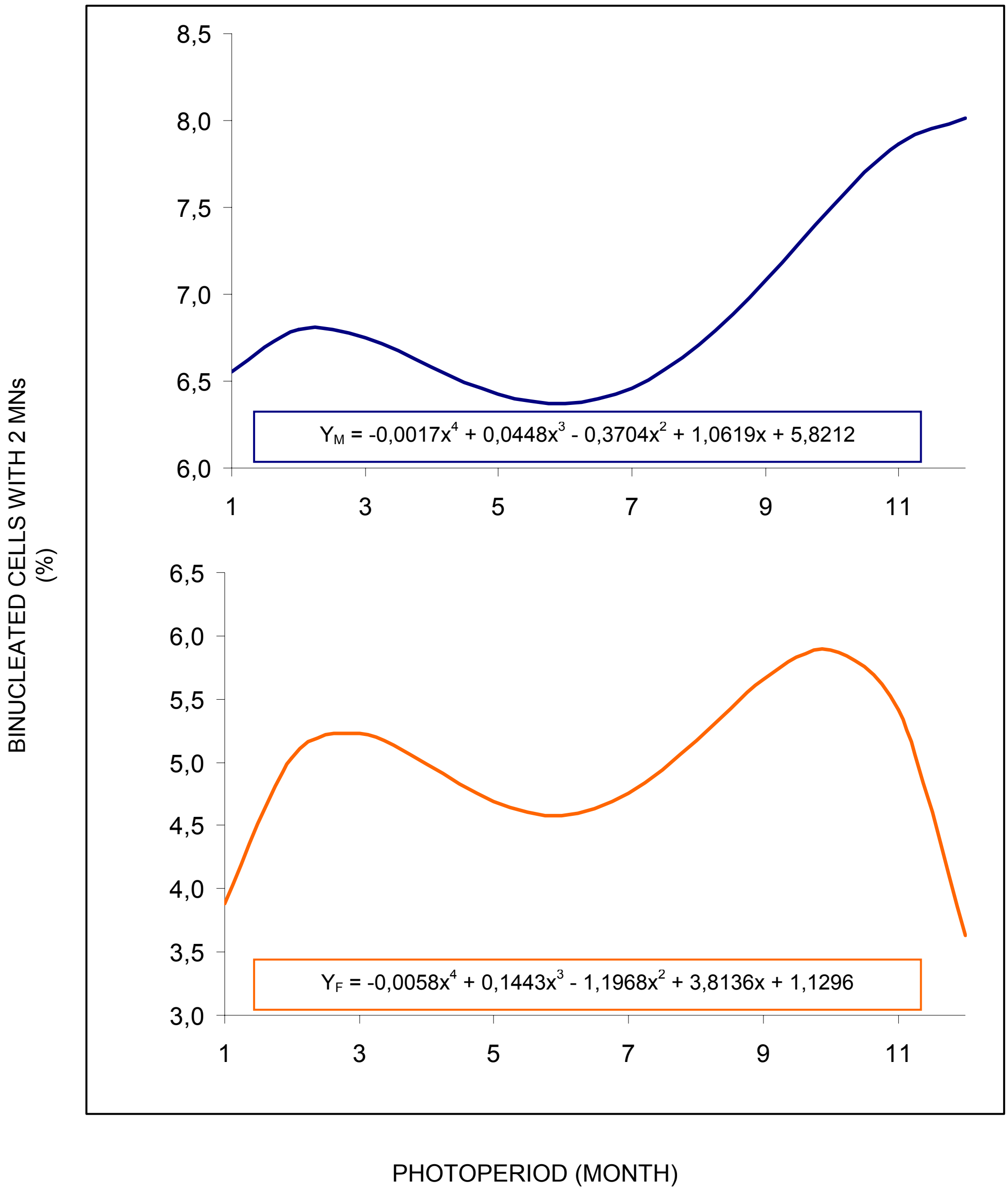


Fig. 2. Course of the percentage of cells binucleate with 2 in operation MNs of the photoperiod (month). I compare male/female



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CRC *LOCUS* SCREENING IN SOME ITALIAN PIG ANCIENT AUTOCHTHONOUS GENETIC TYPES (AAGTs). PRELIMINARY RESULTS

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SUMMARY - CRC (*Calcium Release Channel*) or RYR1 (*Ryanodine Receptor 1*) *locus* is characterised by two allele forms: *N* (dominant or *C* allele) and *n* [recessive mutated allele, also known as *'halothane sensitivity'* allele (Hal^n) or *T* allele]. In pig, Hal^n presence in homozygous condition is responsible for PSS (*Porcine Stress Syndrome*), which, among the other things, causes PSE (*Pale Soft Exudative*) myopathy. PSE meat is not suitable both for fresh consumption and transformation. In order to individuate the possible presence of mutated allele and its incidence in some ancient autochthonous genetic types (AAGTs), a genetic screening was performed on a total sample of 327 pigs belonging to Casertana (CT), Calabrese (CA) and Nero Siciliano (NS) AAGTs. The results of typification evidenced a higher incidence of mutated allele in CA (22%) in comparison with NS (8,7%) and its absence in CT AAGT.

Key words: point mutation, exudative myopathy, ancient autochthonous genetic type, PCR-RFLP.

INTRODUCTION

Any productive chain based on utilization of farm animal has its bases on the relationship between '*biology*' and '*technological poiesis*'. Similarly to other poietic processes (galactopoiesis, oopoiesis, etc.), myopoiesis, is a complex phenotypic manifestation influenced by interaction among genetic and environmental factors (epigenetic factors) (Matassino *et al*., 2005). Meat production aptitude may be estimated on the basis of molecular parameters both at individual and product level through the integration among the several branches of 'omic' science [structural genomics and functional genomics (trascriptomics, proteomics, lipidomics, glycomics, flavouromics, metabolomics)]. CRC or RYR1 is a '*candidate'* DNA segment encoding protein (gene) of high interest for influencing some qualitative characteristics of pork. This DNA segment is recognized as one of the major factor responsible for the 'meat quantity-meat quality' genetic antagonism (Sellier and Monin, 1994).

Up today 31 point mutations have been identified in human RYR1 DNA segment, mapped on chromosome 19, responsible for malignant hyperthermia (MH); 24 out of 31 mutations (77,42%) have been detected in regions including 37% of polypeptide/s coding region of RYR1 DNA segment (Galli *et al*., 2006). In pig, CRC or RYR1 DNA segment maps on the short arm of chromosome 6 and has two allele forms: *N* (dominant or *C* allele) and *n* [recessive allele, also known as *'halothane sensitivity'* allele (Hal^n) or *T* allele]. The presence of two allele forms is due to a point mutation [C (citosine) —>T (tymine) substitution] at position 1843 in the 134 bp exon, responsible for Cys (cysteine) —> Arg (arginine) substitution at position 615 in calcium release channel protein (Fujii *et al*., 1991; Brenig e

Brem, 1992); in the muscle fiber this substitution induces anomalies in the calcium flow regulation at sarcoplasmic reticulum level (ionic calcium deregulation), which in pig species is manifested as PSS (*Porcine Stress Syndrome*) or pig malignant hyperthermia. Meat of PSS animals displays PSE myopathy, which, altering some meat characteristics (pH, water holding capacity, texture of muscle tissue, colour, etc.), makes meat not acceptable both for fresh consumption and transformation.

PSE anomaly starts suddenly after slaughter in association with a rapid muscle pH decline caused by accelerated glycogenolysis. Muscle pH, normally close to neutral value in live animal, may decrease to values less than 5.8 ÷ 5:7 depending on the muscle, causing both muscle protein denaturation with subsequent decrease of water holding capacity and microbic proliferation responsible for putrefactive and degradation processes limiting meat preservability.

As known, the identification of this point mutation has allowed the setting up of a *'genetic prophylaxis'* test employed for the first time by Russo *et al*. (1993). This test is irreplaceable for the following reasons: (i) it allows to identify the mutated allele presence in 'heterozygous carrier' *Nn* or *CT*), which, silmilarly to homozigous subject (*NN* or *CC*), is not reactive to phenotypic halothane test due to recessive nature of mutation; and (ii) incomplete penetrance of CRC or RYR DNA segment: some subjects (10% incidence), altough bearer of mutated allele in homozigous condition (*nn* o *TT*), don't express the proneness to PSS syndrome, so that they escape to halothane test (Ollivier *et al.,* 1975; Webb *et al*., 1987; Otsu *et al.,* 1992). Up today, the genetic test is routinarly employed in cosmopolite genetic types; AGTs/AAGTs are under characterization. The present paper, in the context of a broad plan of productive aptitude valorisation of AGTs/AAGTs for obtaining 'labeled typified local product (LTLP)', is aimed to individuate both the possible presence of mutated allele and its incidence in some ancient autochthonous genetic types.

MATERIAL AND METHODS

Genetic *screening* was carried out on: 120 CT, 104 CA e 103 NS reared at Experimental Farm of ConSDABI, ARSSA, as well as at 14 farms distributed in Sicilian geographical area of Monti Nebrodi (Communes of Alcaralifusi, Brolo, Caronia, Longi, Mirto, Sanfratello and Tortrici). Genotype determination was performed by *PCR-RFLP* (*Polymerase Chain Reaction – Restriction Fragment Lenght Polymorphism)* test (Russo *et al*., 1993) from genome DNA extracted from blood or hair. Restriction analysis was carried out using CfoI and AspI enzymes and visualizing restriction pattern obtained on 3% agarose gel (NuSieve 3:1) by *multipurpose gel and blot imager Typhoon* 9210.

RESULTS AND DISCUSSION

Results of typification, valid within the limits of the observation field, evidenced as follows: (i) allele frequency (Fig. 1): (a) *n* (or *T*) allele results absent in Casertana AAGT while its frequency is 0.09 in Nero Siciliano AAGT and 0.22 in Calabrese AAGT; (b) *N* (or *C*) allele results to be present with a frequency equal to 1, 0.91 and 0.78 in CT, NS and CA, respectively; (ii) genotype (Fig. 2): (a) all 120 subjects tested of Casertana AAGT belong to *NN* (or *CC*) genotype; 85 out of 103 (82.52%) subjects of Nero Siciliano belong to *NN* (or *CC*) genotype; 58 out of 104 (55.77%) subjects of Calabrese, result to be genotypically *NN* (or *CC*) ; (b) *Nn* (or *CT*) genotype results to be equal to 17.48% (18subjects) in Nero Siciliano AAGT and to 44.23% (46 subjects) in Calabrese AAGT; (c) no *nn* (or *TT*) genotypes result to be present in the three AAGTs examined; and (iii) the two AAGTs CA and NS diverge significantly ($P<0.01$) from Hardy Weinberg equilibrium due to an excess of homozygotes.

The aforesaid results confirm those of two previous research (Matassino *et al.*, 2000 and 2007a) obtained on a smaller sample (28 and 110 subjects of CT, respectively; 33 NS in Matassino *et al*., 2000). Extending the size of the tested subjects, with particular reference to Calabrese AAGT, an increase of heterozygote subjects percentage ('healthy carrier') was observed.

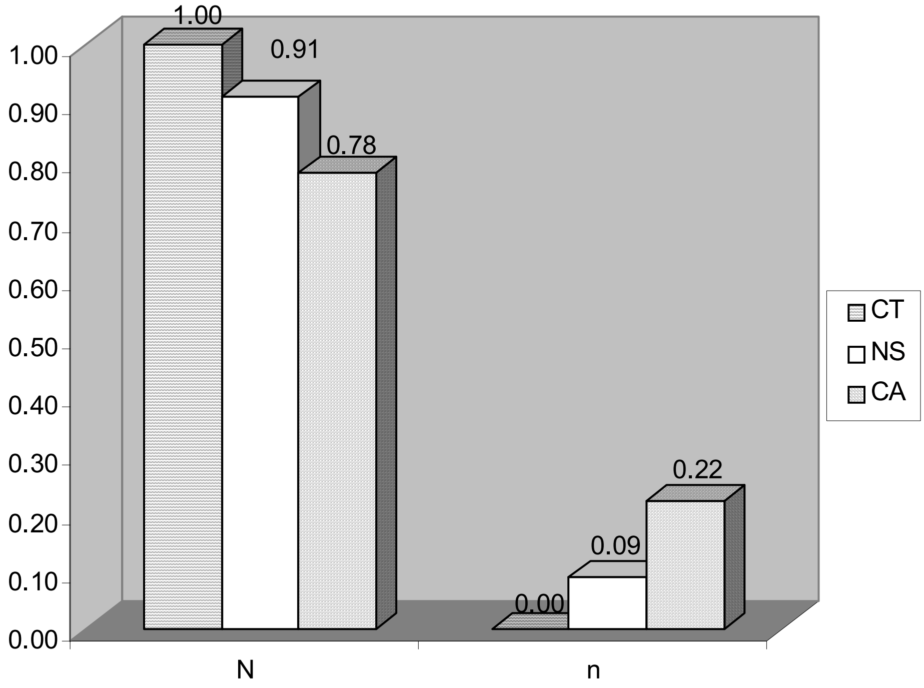

Fig. 1. Allele frequency in the three AAGTs examined.

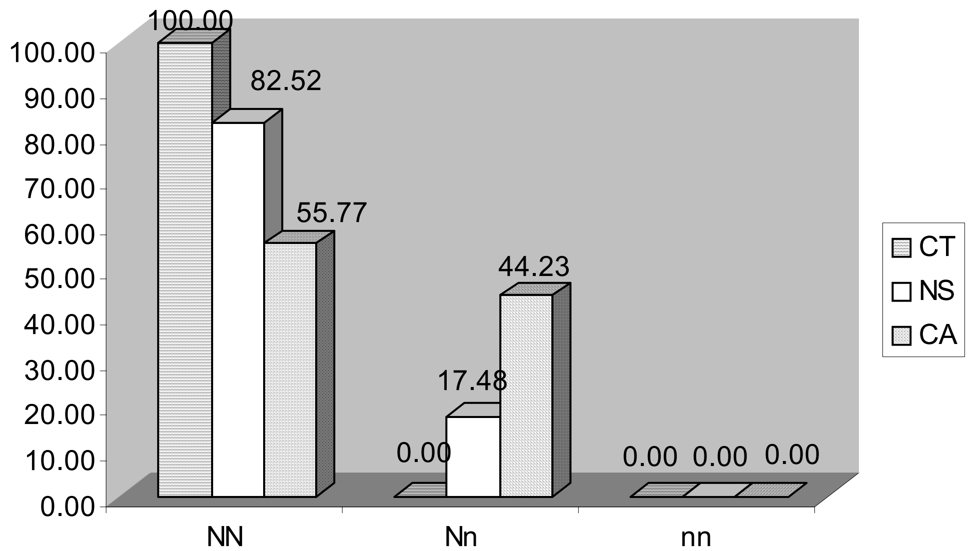

Fig. 2. Percentage frequency of genotypes in the three AAGTs examined.

The presence of mutated allele in some AAGTs might be explained by some attempts to cross them with cosmopolite genetic types , such as Pietrain for Nero Siciliano (Russo *et al.* 2004) .

For Nero Siciliano AAGT, mutated allele carriers tend to be concentrated in the same farms, suggesting an unconscious attempt of farmer to favour heterozygous genotype due to its better productive performances especially in terms of leaness. Indeed, Webb and Jordan (1978), Santoro *et al*. (1982), Tor *et al.* (2001) evidenced that heterozygous (*Nn* o *CT*) and recessive homozygous (*nn* o *TT*) provide a higher percentage of lean cuts and ham when compared with dominant homozygous (*NN* or *CC*) subjects ; the better quantitative performances linked to the presence of mutated allele would be also supported by a higher incidence of *n* allele in Pietrain pig (Webb *et al*., 1982; Russo *et al.*, 1996), a breed characterised by more developed muscle (Zhang *et al.*, 1992). *n* allele doesn't influence only meat quality, but it would affect also other economical traits, such as some reproductive performances; indeed, some researchs, although contrasting, show a trend of *Nn* sow to farrow medially heavier and larger litter per single delivery when compared with *NN* and *nn* sow; this higher reproductive efficiency (heterotic effect?) of heterozygote would be result higher than that observed comparing between them the two homozygous genotypes (*nn vs NN*) (Webb *et al*. 1982; Stadler, 1995; Stadler *et al.* 1997; Cechova *et al*., 2007). The peculiar behaviour of heterozygote solidifies also for the number of DNA segments having a different expression level; indeed, from a research of Russo *et al*. (2006) emerged that in Pietrain pig the number of differentially expressed DNA segments is equal to 766 in *Nn* genotype carriers and only 288 in *NN* subjects. This different behaviour of *Nn* genotype, in comparison with *NN* , could be explained by the possible 'master' role of *n* allele able to activate, according to 'tumble' mechanism, other DNA segments influencing several phenotypic manifestations (Matassino *et al*., 2007a).

The superiority of some reproductive and productive performances of heterozygous individual is not accompained by an improvement of meat quality traits,such as pH post mortem variation and electrical conductivity (Sellier e Monin, 1994; Tor *et al*., 2001).

CONCLUSIONS

The genetic test is a tool useful for MAS (*Molecular Assisted Selection*) in order to evidence at birth carriers of mutated allele both in heterozygous and in homozygous condition to eliminate them both from reproductive circuit and from meat production circuit. The presence of subjects not carriers of *n* allele (*NN* o *CC*) is a fundamental requisite for obtaining products candidate to quality label [Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and traditional speciality guaranteed (TSG)]. Testing assumes high relevance for small autochthonous populations since that the presence of 'healthy carriers' would cause the rapid fixing of mutated; furthermore, the distruction of PSE meat would behave negative consequences on enterprise economy .The diffusion of mutated allele also in autochthonous genetic types indicates the need to broaden typification both AGT/AAGT number and number of tested subjects within AGT/AAGT.

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SESSION 2
SANITARY APPROACHES IN THE FARMING SYSTEMS

PIG PARASITES: A SHORT ITALIAN HISTORY

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SUMMARY – More than one century (1898-2007) of literature was collected according to a specific form. In this period 170 papers was produced. Even if the number of papers remain very low a trend of growing interest was observed. Among the different parasites the interest was pointed mainly on gastro intestinal ones (31%), followed by *Ascaris suum* (11%). The half of scientific production is concerning epidemiological angle, the therapeutic approach to the parasitic diseases is also treated (11%) and an interesting 14% has an educational approach consisting in reviews. The geographical distribution only partially reflects the importance of regional pig industry; Lombardy (15%), Emilia-Romagna (13%), Sicily (13%), Tuscany (10%) and Umbria (9%). Is surprising this gap of national scientific production on pig parasite considering the big effort of innovation of swine breeding that transformed in few years the ancient domestic mammal in a technological unit meat producing.

Key words: parasites, swine, history, meta-analysis.

INTRODUCTION

Italy, express today a natural vocation for pig breeding and swine products transformation. The geographical position of this country it's perfectly settled in the centre of Mediterranean basin where many countries express similar peculiarity. The first step to understand the relationship between pigs and parasites is to look back to the historical literature on the topic.

The literature of nearly one century (1882-2007) was collected and organized according to a specific form (Fig. 2). Each paper was read and assigned to different categories. The first paper that correctly fit in our form (where all information were available) is that of Perroncito (1882) and the last of Crotti et al. (2007). In this temporal space 170 papers were produced; with different concentration by year (Fig. 1). For instance, two "black holes" (1%) with only one paper were found in the decades 1910-1919 and 1920-1929 in opposition to the "golden decennium" found in 80's. Even if the number of papers remains very low, a trend of growing interest was observed starting to 50's following the revolution of pig farm industry. The peak (1980 – 1989) coincides with the born of new molecolar family of endectocides (macrocyclic lactones) that give an industrial booster to the research.

The geographical distribution, only partially reflecting the development of regional pig industry is: Lombardy (15%), Emilia-Romagna (13%), Tuscany (10%), Umbria (9%), Sardinia and Apulia (7%). The percentage (13%) of Sicily, where pig industry is not strategic it is wondering, but it is due to the big scientific effort done on local pig breeds (Figs 3 and 4).

Some paper was realized without a strict regional definition and was grouped with different labels (Italy, Northern Italy, etc.). Another key of lecture of such a distribution should be linked to the presence of a parasitological school in the involved area.

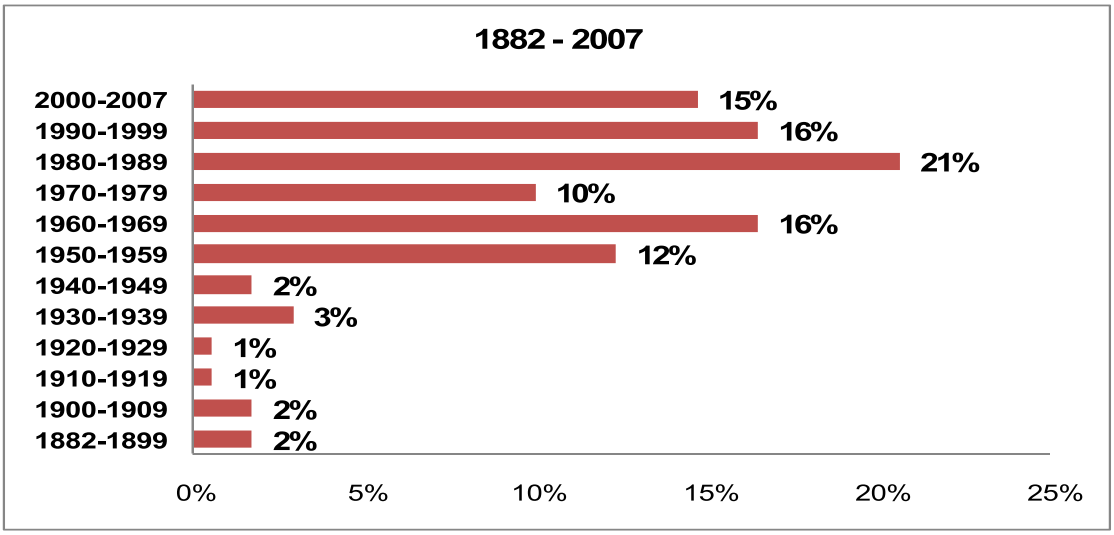
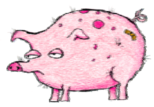


Figure 1. Scientific production by years organized by decades.

Title____

Author ______________________________ Year__________



Published on ____________________________ Vol./Pag. __________

Type of papers: 1) Clinical case □ 2) Original research □
3) Review article □ 5) Research project □

Operating procedures: 1) Faecal exams □ 2) Necropsy □ 3) Serology □
4) Skin exams □ 5) Biotechnology □ 6) Hematology □
7) Parasite morphology □ 8) Zootechnical parameters □ 9) Others □

Alive □ Dead □

Monothematic □ Polithematic □

Topic ____
Pointed out parasite ____________________________________
Experimental data available? ⊥ ? no □

Research geographic area: ________________________________

Field of study: 1) parasites morphology □
2) anatomy - pathology □
3) epidemiology □
4) zootechny / zooeconomy □
5) diagnostics □
6) teraphy □
7) parasites diseases □
8) immunology – immunopathogenesis □

Note:____

Figure 2. Data collection form

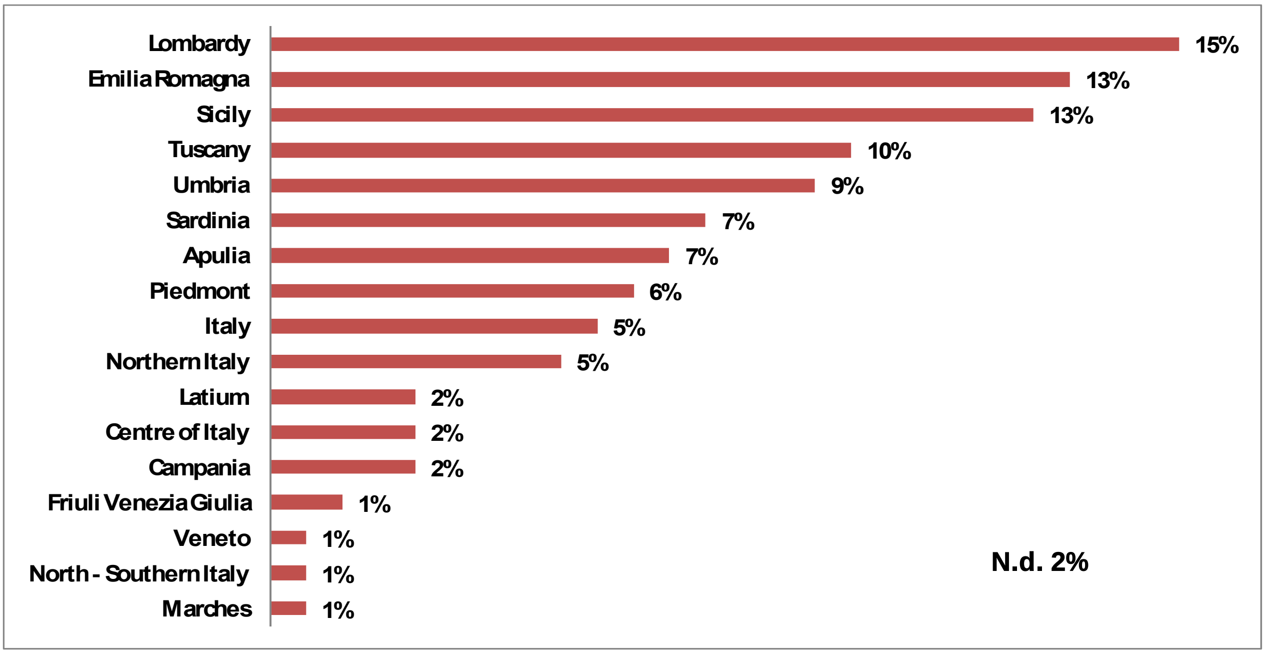


Figure 3. Scientific production by geographical area

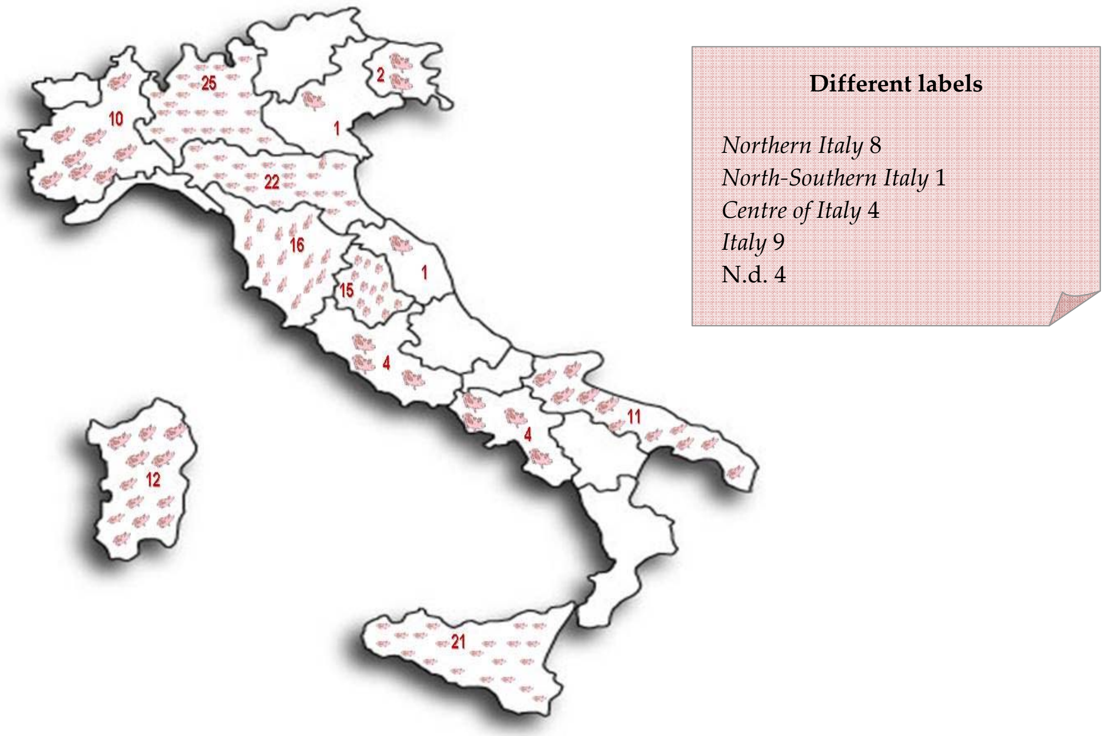


Figure 4. Scientific production in different Italian regions.

In the figure 5 are resumed the parasites found in italian pigs in the considered period. 31 genera and 38 species belonging to protozoa (12), flatworms (6), roundworms (16), acantocephala (1) and arthropods (3). The lecture key of the list should consider the long time investigated and the development of the parasitological interest linked also to the modern pig breeding systems. Many parasites are an heritage of the past, someone has changed his name and for this reason we have considered right highlight the parasites who are still present in the modern pig breeding. For practical purpose in the figure 6 are shown the different habitat of the above mentioned parasites. Despite all that, the other parasites still remain in the wild boar that is spread all over in Italy.

PROTOZOA	HELMINTHS			ARTHROPODS
	FLATWORMS	ROUNDWORMS	ACANTOCEPHALA	
Amebe	*Brachylaemus suis*	*Arduenna strongylina*	*Macracanthorhyncus hirudinaceus*	
Babesia perroncitoi	*Cisticercus cellulosae*	*Ascaris suum*		*Eristalis tenax*
Babesia trautmanni	*Cisticercus tenuicollis*	*Ascarops strongylina*		*Haematopinus suis*
Balantidium coli	*Dicrocoelium dendriticum*	*Globocephalus molin*		*Sarcoptes scabiei*
Cryptosporidium parvum	*Echinococcus granulosus*	*Hyostrongylus rubidus*		
Eimeria debliecki	*Fasciola haepatica*	*Metastrongylus sp.*		
Eimeria polita		*Oesophagostomum dentatum*		
Eimeria scabra		*Oesophagostomum lungicaudum*		
Eimeria spinosa		*Physocephalus sexalatus*		
Giardia sp.		*Simondsia paradoxa*		
Isospora suis		*Strongyloides ransomi*		
Toxoplasma gondii		*Strongylus paradoxus*		
		Trichinella spiralis		
		Trichuris suis		
		Trichuris trichiura		

Fig. 5. List of the parasites found in Italian pigs in the considered period.

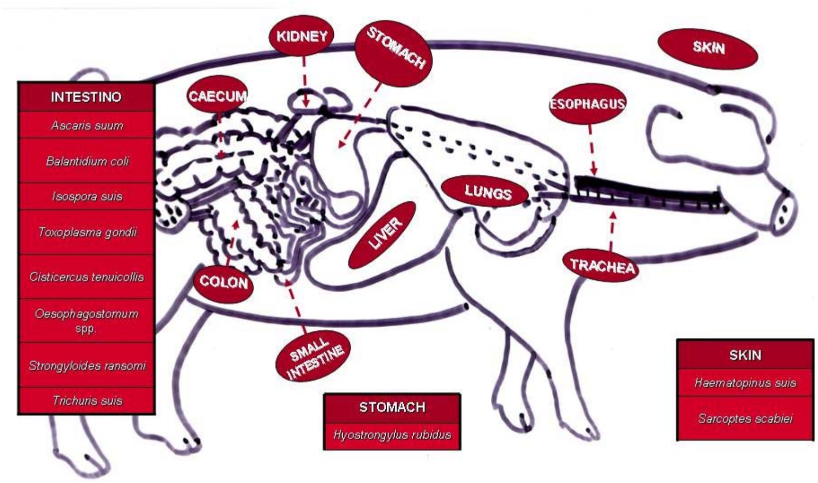


Figure 6. The habitat of the most important parasites in the modern pig breeding

The 62% of the papers (Fig. 7) has delt with more than one parasites while the remaining 38% is referred to only one. This should be linked to the historical period that correspond to the born of modern parasitology and to the morphological approach linked to the pathological description.

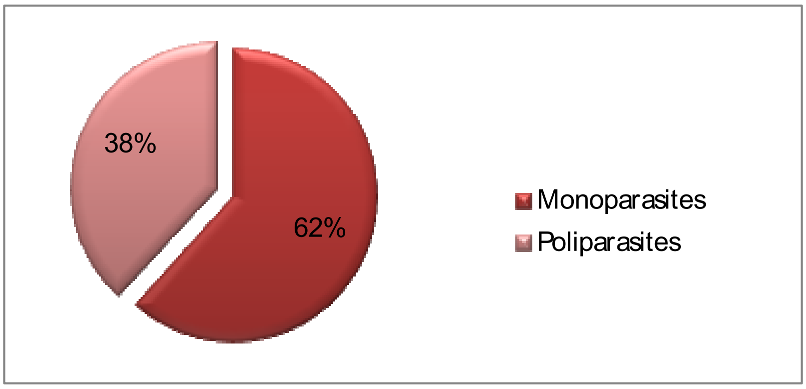


Figure 6. The parasites treated in each paper.

The most considered parasites in our historical collection (Fig. 8) are gastro-intestinal ones reflecting their real practical importance. In a “meat producing engine” the digestive apparatus acquires an enormous weight related to their negative influence on the pig performances. In fact, to the helminths (31%) we have added, the protozoa (15%) and *Ascaris sum* alone (11%) with liver lesions (11%).

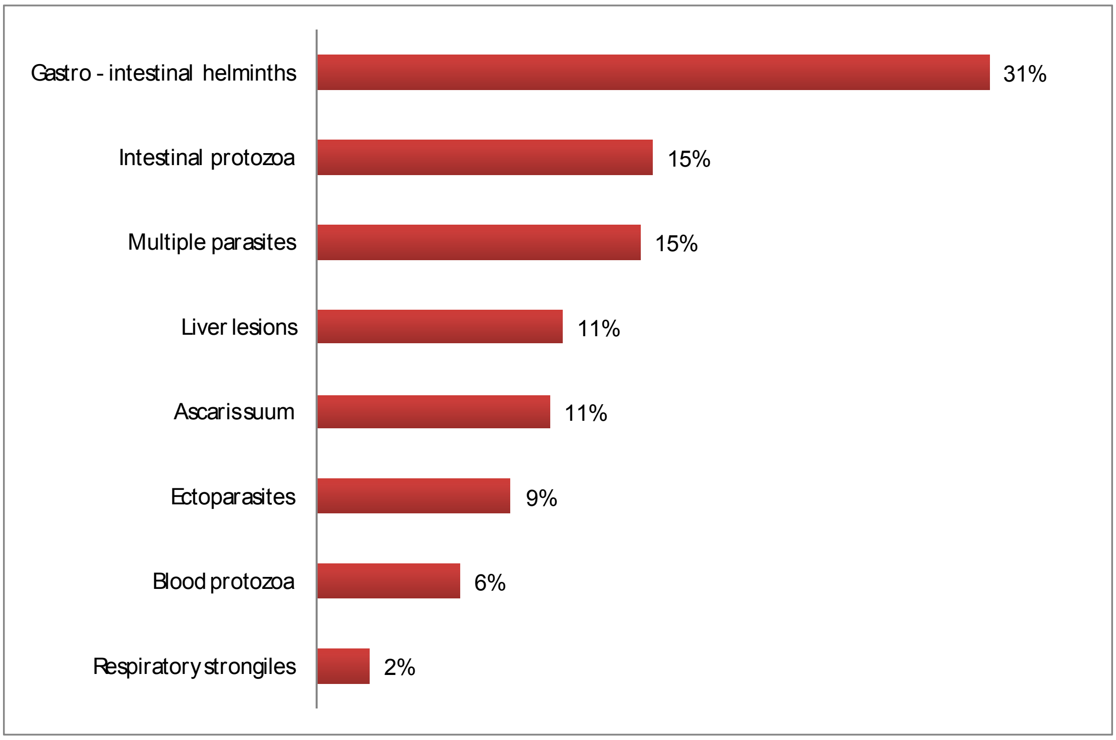


Figure 7. The most considered parasites in the examined collection.

The scientific origin of the papers is shown in the figure 9 where is possible to appreciate that the majority (70%) is specific original papers. The 15% reflects the old approach of description of single case (parasite) while the 14% are reviews with an educational purpose. Only one is the result of a national research project .

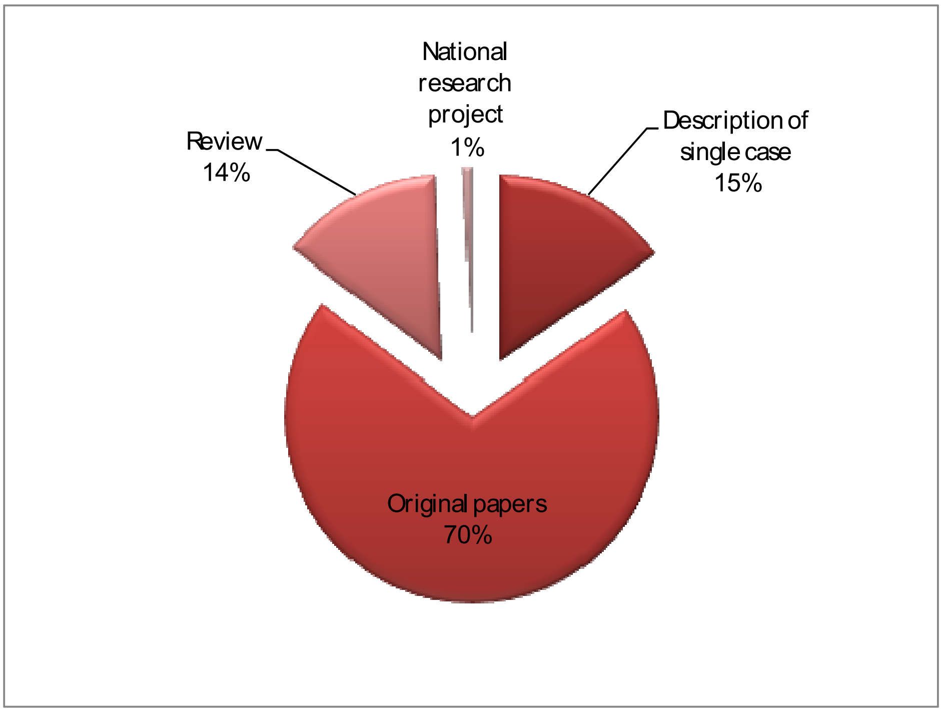


Figure 8. Scientific origin of the papers.

The diagnostical approach to the swine parasitic disease (Fig. 10) privilege a multiple approach (40%) followed by the necroscopical (29%) and coprological one (20%). Serological (7%) and dermatological (4%) appear less treated in relation to their recent use in the laboratory practice.

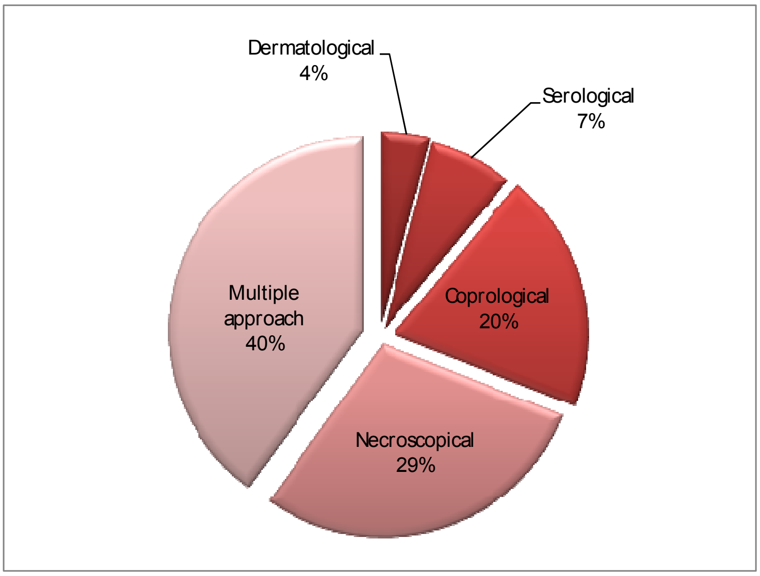


Fig. 9. The diagnostical approach to the swine parasitic diseases.

The different topics in the approach to the swine parasitic diseases (Fig. 11) absolutely prefer the epidemiological angle (64%). A scarce weight receive, for instance, zooecnomy (3%) that should represent the pivot of a modern approach to animal disease.

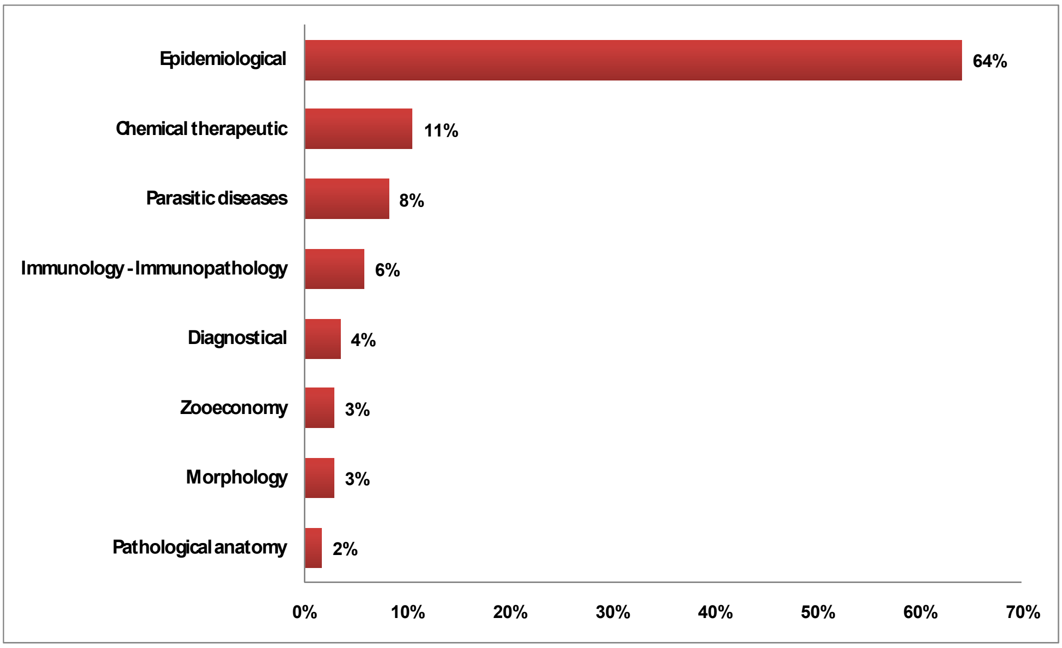


Figure 10. The main topics in the swine parasitic diseases.

The observed gap in national scientific production on pig parasite is surprising if related to the big effort to renew swine breeding that transformed in few years the ancient domestic mammal in a technological unit meat producing. Parasitic diseases are unfortunately “snubbed” by the Italian pig technicians indeed almost than one third (32%) of the scientific paper produced and collected was just published in local forgotten congress proceedings very hard to find. The pig staff in recent years are interested on therapy only, with lack of attention about hygienic prevention techniques. As consequence, new trends that would come back us to wild and natural pig breeding techniques, unfortunately will crash with a lot of parasites. The modern pig breeding system, has unintentionally taken away spaces to free living stages of parasites. This new pig farm set up (“plain air”) will allow parasites to high-handedly return and it will find us absolutely unprepared.

ACKNOWLEDGEMENTS

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The literature is available directly from the authors.

ADAPTATION ABILITY OF NATURAL IMMUNE SYSTEM IN CINTA SENESE SWINE AND COMMERCIAL HYBRID BREEDING PIGS TO OUTDOOR REARING

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SUMMARY-Some non-specific immunity parameters (serum bactericidial activity, serum lysozyme and total haemolitic complement) were evaluated in different pig breeding systems to verify how these could influence the non-specific immunity system. A suitable utilization of such parameters could be a useful tool in the breeding herd management.

Key words: Cinta senese, Hybrid pigs, Outdoor, Natural immune system

INTRODUCTION

Evaluation of welfare in breeding animals is one of the main topic of both the European and national legislation. Such regulations have the aim to assure acceptable welfare levels along all the stages of the rearing cycle, to limit environmental stress overloading the body control and regulation systems and reducing their efficiency (Broom, 1997). Welfare evaluation implies some behavioral or rearing indicators (productive, physiological, pathological).

Whenever a stress status becomes chronic, all the non adaptive immune system is involved, thus resulting in evident conditioned pathologies. This innate part of the immune system is easily influenced from environmental stress. In our previous works we evaluated the main innate immune parameters in traditional pig breeding. In this work we monitored the same parameters in an intensive breeding herd on outdoor growing and finishing, and in an outdoor pig farm just to evaluate whether and to what extent the breeding system may affect the non adaptive immune system.

MATERIAL AND METHODS

The study was conducted on three different breeding herds:

- An outdoor *Cinta Senese* farm. The herd, located on an hill area, had a population of twenty productive sows. The breeding stocks was host in a nearly one hectare paddock with a kennel. The sows were kept in separate indoor pens inside a hut with a large veranda. The piglets, weaned at nearly thirty five days of age, were moved to a separate one acre paddock. At twenty five – thirty kgs of weight they were moved into another paddock (prefattening) and later on (roughly sixty kgs) in a wider paddock with a large portion of wood, where they remained till the slaughter weight. Every department was working on “all in/all out” basis, apart from the breeding stock.

- Traditional indoor pig farm with 200 on production sows. Pregnant sows were reared in small group pens or in individual cages. The building was naturally ventilated without any heating system. The farrowing houses had traditional raising farrowing pens with steel slatted floor. Under the sow belly there was a large concrete stone; ventilation and heating were electronically controlled. Weaner accommodation was made of a large roof covering a series of kennels. These kennels made with polyurethan isolated panels (sandwich) were straw bedded and had an external paddock. The finishing accommodation was made of a circular building (roundhouse) internally split in different sectors with an external concrete slatted paddock each.

- Traditional intensive indoor pig farm. The herd was composed of hybrid commercial sows, producing self replacement gilts. The farm was organized on a multi-sites basis (three sites production: breeding herd, nursery and fattening unit). The fattening unit had rooms dimensioned on the pig number receiving each week to adopt all in / all out policy, allowing washing and disinfection procedures. Each pen had complete slatted floor and internal temperature was properly maintained by a suitable heating system. A forced ventilation system was installed.

Sampling. A total of ninety blood samples, thirty for each breeding were collected. Blood was collected at slaughter using proper vacuum tubes and it was immediately sent (in a freezer +4° C) to the laboratory, centrifuged at 2000 rpm for 15 min. Collected sera were distributed in several aliquots and kept at - 80° C until tested.

Serum lysozyme, serum bactericidal activity, haemolytic complement activity were investigated. Lysozyme is a strong antibacterial enzyme carrying out a synergic action with immunitary humoral response and factors of the complement. Its determination leads to monitor the functionality of the macrophages haematophage system and it is indicative of ongoing inflammation. Serum lysozyme was measured by the lyso-plate assay (Osserman and Lawlor, 1966), carried out at 37° C for 18 h, in a humidified incubator. The method was based on the lyses of *Micrococcus lysodeikticus* in 1% agarose. The diameter of the lyses zones was determined using a measuring viewer and it was compared with the lyses zones of a standard lysozyme preparation. Its concentration value was expressed in µg/ml.

Serum bactericidial activity is a major parameter for evaluating the activity of the non-specific immune system. The capacity of the serum to inhibit bacteria growth is assessed by the presence of complement factors and moderate concentrations of natural antibodies directed towards more diffused environmental bacterial agents, mainly *enterobacteriacee*. Serum bactericidal activity was monitored according to a previous method validated for bovine (Amadori et al., 1997) Its concentration is expressed in percentage.

The haemolytic complement assay provides indications on the defence mechanisms of the animal that contemplate activation of the complement system. A test showing complement deficiency is of great help assessing the risk of infection or the severity of ongoing pathologies. Haemolytic complement assay (Barta V. et al., 1993) was carried out in microtitre plates; a volume of 25 µl of a 6% suspension of rabbit red blood cells was added to pig serum serially diluted in Veronal buffer. The extent of haemolysis was estimated by measuring the optical density of the supernatant at 550 nm. Its concentration is expressed as CH50.

RESULTS AND DISCUSSION

Serological test results are summarized in Table 1 and figures are expressed as the mean values ±SD established over 30 animals for each group investigated. Significant differences on serum bactericidal activity and total serum lysozyme values between classic indoor versus outdoor pig farm were recorded, while statistical differences between the two outdoor farms were not seen.

The parameter values obtained differ from previously reported data (Moscati et al, 2003) mainly for serum bactericidal activity (> 40 %); This result could be due to a different environmental condition in the farms. Serum bactericidal activity values recorded in both outdoor farms are lower then traditional indoor farm (natural antibodies levels decreasing). At the same time total serum lysozyme levels are increased although still within the normal range. This fact could be due mainly to a granulocytic de-granulation rather then an increased activation of monocitary – macrophagic system.

CONCLUSIONS

The use of non-specific immune parameters could be useful to have information on the animal capacity to adapt to the living environment. This capacity could be influenced also by the genetic patrimony. Further investigation on the different environmental conditions might highlight which breeds are more suitable for the different breeding typologies.

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Table 1. Non-specific immunity parameters comparison between the breeding herds monitored (Avgs ±SD)

	Reference values	Intensive traditional rearing	Commercial hybrid outdoor rearing	Cinta senese outdoor rearing
Serum Lysozyme µg/ml.	>1- <3	1.48 ± 0.1 A	2.82 ± 0.6 B	2.58 ± 0.2 B
Haemolytic Complement Activity CH50/150 µl	>80	83.9 ± 1.83	90.7 ± 2.81	90.9 ± 2.19
Serum Bactericidal %	>40	54.3 ± 2.1 A	31.2 ± 1.21 B	30.8 ± 2.17 B

A, B: $P<0.05$

IDEAL APPROACH TO HEALTH MANAGEMENT IN AN OUTDOOR PIG BREEDING FARM: A CASE STUDY ON THE UMBRIAN APPENNINE

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SUMMARY - Outdoor pig production have to respect all the health management principles as an indoor farm. "One way" pig flow and "all in/all out" policy represent the mainpoints. A 32 production sows outdoor farm, located on Umbrian Appenine, adopted the good production and health management practices as already experienced in other countries (U.K and French Bretagne). Productive performances obtained in the last years are highly competitive versus indoor pig farms results. The health status of the breeding stock and the progeny is excellent. The fully respect of the mainpoints of health management system in an indoor pig farm represents the key for the productive success and product quality assurance.

Key words: outdoor, pig, health, performances

INTRODUCTION

Rational outdoor pig production represents an innovative and relatively unusual approach to pig keeping within Italian animal breeding contest. It is a system where breeding sows are reared extensively outdoor for whole their productive cycle. In its simplest version it was implemented along the 20th century, where it took its origin from an idea of Richard Roadnight, an Oxfordshire agriculture. At the beginning of '50ties years he had the intuition to get a more harvest from his agrarian culture program.

Using "Roadnight system" (3) the sows were reared in large groups, in wide paddocks where they farrowed twice a year and their progeny were weaned at 8 weeks of age. British Saddleback was the main genetic breed used, due its evident roughness attitude and the backfat thickness make it particularly resistant to external environmental conditions. Along the years such breeding system was even more evolving and achieving always much more consents and today it represents a large percentage of pig farms in many European countries.

Outdoor pig production could represent a suitable, valid and remunerative alternative to the indoor production, in Italy too (1). This system can offer some advantages like animal welfare respect, lesser capital investment (especially in the starting phase), improvement of soil quality (due to the natural fertilization), optimize the human labor and the equipments. It is included in a rotational five years agriculture plan and it is able to produce a "continuous cash flow" , impossible to obtain with the normal cultures only (3).

The market demand for always more leaner meat has brought to a gradual substitution of ancient genetics with modern commercial hybrid Duroc derived populations. The Outdoor pig production even if is able to offer natural living conditions has to respect all the fundamental principles where health management and productive performance optimization are based.

They are:

1. Ideal herd census respect: breeding sows splitting in order their progressive parity (Table 1) to the aim to assure maximum productive performances from the breeding stock and the herd immune stability (4);

2. Body Condition Score utilization on the breeding sows to evaluate and assure their suitable feeding along their physiological productive phases (3);

3. “One Way” productive flow respect for having herd immune stability and to prevent reinfections (2);

4. “All in / all out” policy adoption to decrease the potential pathogens levels.

Table 1. Ideal herd census (M. Sensi, 1993)

	Max number parity/sow (%)							
Prog.ve parity	2	3	4	5	6	7	8	9
1	54,1	38,9	31,4	26,9	24,1	22,1	20,6	19,5
2	45,9	33,0	26,6	22,9	20,4	18,7	17,1	16,6
3		28,1	22,7	19,5	17,4	16,0	14,9	14,1
4			19,3	16,6	14,8	13,6	12,7	12,0
5				14,1	12,6	11,5	10,8	10,2
6					10,7	9,8	9,1	8,7
7						8,3	7,8	7,4
8							6,6	6,2
9								5,3

MATERIAL AND METHODS

On the Umbrian Appennine it exists an interesting example how the use and the rigorous respect of the fundamental principles regulating health and productive management of pig breeding can result in good productive performances quite competitive respect the best indoor reality.

This farm has 32 breeding productive genetically Duroc derived sows. Since the start Duroc boars were bought from the market while all the females were on farm produced on outdoor basis to give them much more roughness attitude. A part from the matings (using A.I), done indoor, all the other productive phases are outdoor: farrowing, weaning, growing and finishing till 165 kg (roughly).

Figure 1. Farrowing kennel

Figure 2. Weaners

Each phase, following the already well known multisites principles, are done in distinct electrically fenced paddock, completely separated each other. The breeding herd is managed “in groups” to assure a better planning and much more animal growth uniformity. Such organization allows omogenous pregnant sows groups sharing the same breeding conditions and reducing aggressive phenomena for the competition. These system characteristics are quite interesting. The sows farrow outdoor, without any assistance, in single straw bedded kennels, in presence of adverse climate condition, too (snow).

The piglets are milked since four weeks of age and their weaning weight is nearly 10 kgs/piglet. The sow are ad lib fed through a big hopper where the piglets can eat, too. They start to follow their mother since 7 – 10 days of age.Using this system the sows is in suitable body condition at the its next heat, with the ideal backfat thickness.

At weaning all the weaners are tattooed, castrated, vaccinated and moved in a wide weaning paddock.They are ad libitum fed with phase specific commercial feed. The weaning phase ends when the weaners weight nearly 35 kgs. The next phase, “growing” is done in an other separate paddock were the animals are reared till they weight roughly 100 kgs. All the growers can shelter in a container (roundhouse) able to host 120 pigs. The feed hoppers are numerous to avoid competition and the growers eat ad lib. The pigs are moved in the finishing facilities, in small groups, when they are 100 kgs weighting. In the finishing stage, infact, it is extremely important to build a suitable market carcass then these paddocks are quite wide to allow the grass growth, necessary for animal pasture.

The farm has achieved contract of finishing with some neighbours that make their estates and labour available. This allows “one way “ production flow and “all in/ all out “ policy adoption. The finishing period can vary related to the final meat destination (fresh meat consumption or salami and hams productions). It is important to highlight the presence of an on farm meat shop. The farm can achieve a better income due to this system that offers to the customers the possibility to interact with the farm staff and assure their faithfulness.

We recorded, in this work, the farm productive and reproductive performances obtained since 2000 till the end of 2005 (Table 2). A 2005 year economic balance was then produced making a comparison with an indoor traditional pig farm, as reported in 2005 CRPA leaflet (Table 3).

RESULTS AND DISCUSSION

It is quite evident, from Table 2, how the breeding sows number is continuously increased along the years. The individual sows performances are improved at the same time.

The outdoor pig production as the indoor one is obliged to adopt precise and correct managerial, zootechnical and health strategies. Their perfect integration results in the productive performance optimization from the economic point of view, too (Table 3).

Table 2. On farm productive performances

YEAR	Breeding productive sows	Piglet weaned Sow/year	Pigs sold Sow/year
2000	11	20,6	18,4
2001	14	19,4	19,1
2002	18	20,9	20,5
2003	22	21,3	20,7
2004	25	21,2	21,0
2005	32	24,37	23,1

Table 3. Costs (€/kg l.w.) “Indoor” vs “Outdoor” (2005)

PARAMETER	INDOOR “indoor” source CRPA 2005			OUTDOOR (farm balance 2005)		
	Capo	Kg	%	Capo	Kg	%
Feeding	126,40	0,79	56,8	142,90	0,90	79,10
Labour	33,93	0,21	15,1	23,84	0,15	13,20
Other costs	41,60	0,26	18,7	8,49	0,05	4,70
TOTAL explicit Costs	201,94	1,26	90,6	175,23	1,09	97
Interests and Allowances	21,09	0,13	9,4	8,12	0,05	3
TOTAL COSTS	223,02	1,39	100	183,35	1,131	100
COSTS €/kg		1,39			1,131	

CONCLUSIONS

During the last years public opinion showed an increased sensibility for what concerning animal welfare and Health safety of their products especially meat. The breeder responsability for animals (welfare), for Nature (territory respect) and Human Health (Food safety) is clearly emerging from the actual European community legislation.

The animal welfare is always more achieving sense of importance. Pig production have to measure itself with this new scenario, answering to the E.C. indications. For this reason, outdoor pig production could represent an interesting economic investment, useful marginal areas recovery and to supply the small farmers with an extra income.

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Nero Siciliano pig. A model for the study of unusual pathologies

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SUMMARY - Aim of the present paper is the evaluation of the diseases related to the farming system (*plein air* and intensive) occasionally detected in Nero Siciliano pig, an autochthonous breed, usually farmed in extensive conditions in regional forest areas. 20 subjects coming from an intensive farm and 20 subjects coming from a *plein air* farm were examined at the slaughterhouse. Histopathology was performed on all organs and tissues. In swines farmed intensively findings were as follows: presence of hair-balls (8 trichobezoars and 2 trichophitobezoars) in ten animals; in 50% of the pigs, the liver showed chronic eosinophylic interstitial hepatitis (milk spots); in two subjects steatosis was found; features of vegetative endocarditis were seen in a single animal; the kidney in a pig showed interstitial nephritis and only one animal had alveolar emphysema in the lung. In swines farmed in *plein air*, one subject showed vegetative endocarditis, later related to *Erysipelotrix rusiopathie,* while in fifteen pigs, melanosis at retro-pharyngeal lymph nodes was seen. Findings here reported represent a further contribution to the knowledges of diseases occasionally occurred in Nero Siciliano pig, and let the Authors consider such breed as experimental model for the study of unusual pathologies as well as for the analogies of comparative pathology.

Key words: Nero Siciliano pig, phatologies, farming system, experimental model.

INTRODUCTION

The Nero Siciliano pig is an swine autochthonous breed living in extensive conditions in regional forest areas, whose diseases are still to be elucidated in order to prevalence and incidence. Recently, the *plein air* farming system is considered the best for growing, fattening, lower stress, higher performances and better shelf-life of transformed food (Liotta et al., 2002; Chiofalo et al., 2003). Aim of this study is to evaluate pathological outcomes related to the farming system. Moreover, our findings provide a contribution to the knowledge of organ changes in pigs belonging to such breed reared in an intensive system.

MATERIAL AND METHODS

Twenty subjects coming from an intensive farming system and 20 subjects from a *plein air* farm, both located in the Nebrodi mountains, were examined at the slaughterhouse. All the animals were *post mortem* examined. Histopathological evaluation were carried out in those organs macroscopically involved. Tissue samples were fixed in 10% buffered formalin solution, paraffin embedded, microtome sectioned at 5 μm and stained with Haematoxylin–Eosin (HE).

RESULTS

Intensive **farming system**:

The presence of hairballs (8 trichobezoars and 2 trichophitobezoars) was detected at stomach opening in 10 pigs (Fig.1).

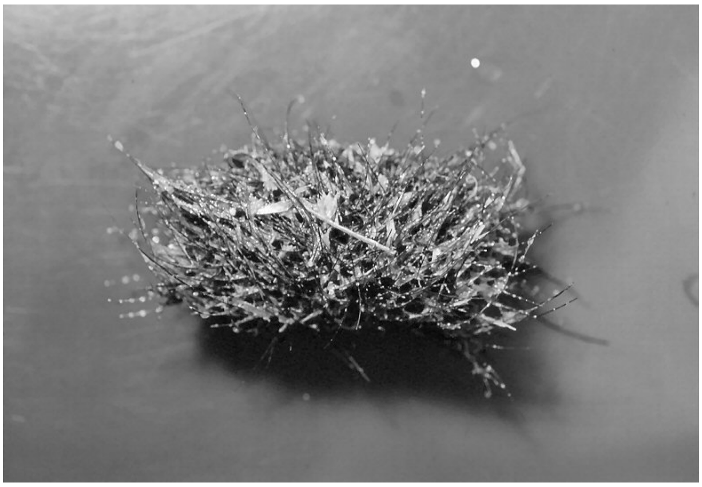

Fig. 1. Hairballs found in the stomach.

The stomach and oesophagus mucosa showed slight hyperaemia, small erosive foci, flattening of mucosal folds with small greyish nodules. The latter were histologically characterized by lymphocytes infiltrate involving the mucosal glands.

In 50% of the examined pigs, the liver showed greyish foci, spread on the liver surface, with irregular and not well demarcated margins indicative of chronic eosinophylic interstitial hepatitis (milk spots); hepatic steatosis was observed in two subjects.

A valvular vegetative endocarditis was seen in the heart of one pig, whereas small sub-endocardic haemorrhages were found in three subjects.

The kidney of one pig showed grey-whitish foci, with capsule thickened and adherent. At the cut surface, the foci appeared as cortical striations, suggestive of interstitial nephritis. In a subject, a single uniloculated congenital cyst was seen; finally, a hypoplasic kidney was observed in only one pig. The lung of one animal showed alveolar emphysema.

***Plein air* farming system**:

One subject had vegetative endocarditis, confirmed microbiologically as an *Erisypelothrix rhusiopathiae* infection (Fig. 2).

Fig. 2. Heart: vegetative endocarditis.

Fifteen pigs showed melanosis at different lymph nodes, mainly the retropharyngeal ones (Fig. 3).

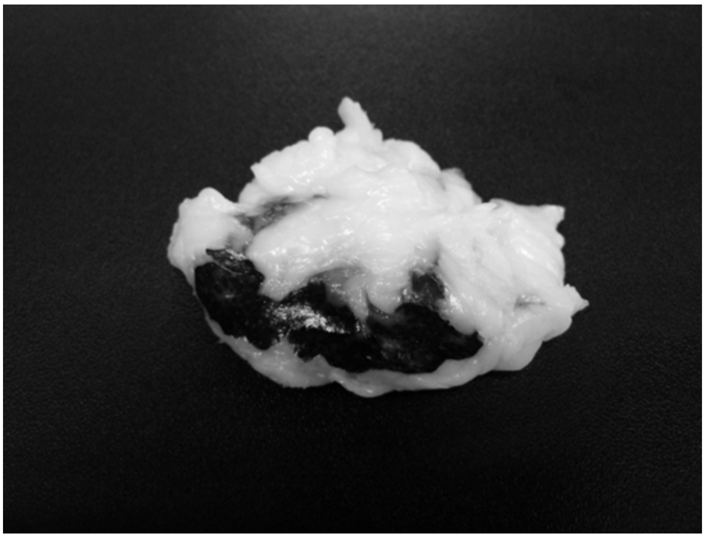

Fig. 3. Retropharyngeal lymph node showing melanosis.

One of these pigs had also melanosis in the fat tissue of the back (Fig. 4).

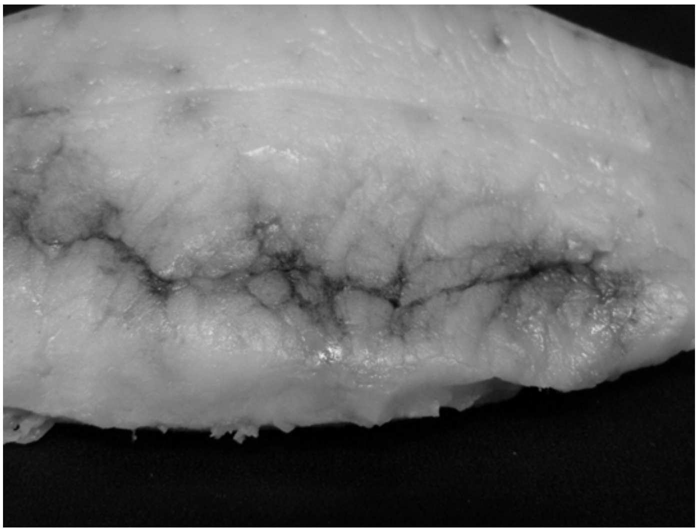

Fig. 4. Fat tissue of the back with melanotic striations.

DISCUSSION

The reported results represent a further contribution to the knowledge of diseases of Nero Siciliano pig among the few existing in literature. Moreover, the comparison of the findings observed in two different farming system let the Authors to consider this breed as a model for the study of unusual pathologies. It is interesting to underline the presence of gastric hairballs in 50% of the examined pigs reared in intensive system. This is an unusual finding in swine (Cagienard, 1970; Clague, 1970; Cooke, 1970; Phillips et al., 1998) and it has never been reported in pigs farmed using extensive methods. As regards the pathogenesis of hair balls it is possible to make some hypothesis (Johnson et al., 1978). The farming system, when not good for the needs of a certain breed, may induce behavioural anomalies able to interfere with the animal health status. In fact, we observed the habit in alive pigs farmed intensively to lick continuously each other, being this behaviour possibly stress-related. In our opinion, according to the macroscopic features, the vegetative endocarditis observed in one intensively farmed pig could be also related to a chronic infections due to *E. rhusiopathie* and the same resulted not strictly related to the farming conditions being observed in one subject for each group. The features of lymphocytic gastritis could be related to *Helicobacter* spp. infection more than to hairballs. Interstitial hepatitis could be due to ascarid worms and it does not represent a new finding considering the diffusion and the frequency of such parasitic disease in swine. Interstitial nephritis has been often related to leptospirosis. Renal cyst is commonly observed in pig, whereas kidney congenital hypoplasia is an interesting finding because of its rarity in swine, and never reported in Nero Siciliano pig to date. Finally, the finding of melanosis in 15 pigs (75%) farmed in *plein air* could represent the effect of acorn ingestion during fattening (Lanteri et al., 2006a and b; Lanteri et al., 2007), more than the hypothesis of tumour regression as already suggested by other Authors (Oxenhandler et al., 1982).

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TRICHINELLOSIS AND EXTENSIVE FARMING SYSTEM: AN ECO-EPIDEMIOLOGICAL APPROACH OF THE SANITARY SITUATION IN INSULAR AREA OF CORSICA

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SUMMARY - In 2004 in Corsica, *Trichinella britovi*, a nematode responsible for Trichinellosis in humans, was detected in slaughtered 10 pigs and 1 fox, whereas this Mediterranean island was considered as free from *Trichinella*. As the extensive farming system of pigs in Corsica often leads to contacts with wild animals, when studying *Trichinella* epidemiology we have to take into account the possible inter-species transmission of parasites between domestic and wild animals. Based on the mobilisation of local operators and especially hunters, the aim of the study was to analyse host reservoirs and sentinel animals which are wild boar, *Sus scrofa*, and foxes, *Vulpes vulpes*. After one hunting season, 670 samples of wild boars' diaphragm have been collected but revealed no positive. Another sampling season will be necessary to obtain an estimation of the prevalence. The final results will be compared to those obtained by the study of fox samples, also under way and presented here.

Key words: Trichinellosis, wild boar, fox, epidemiological surveillance

INTRODUCTION

Trichinellosis is a parasitic zoonosis due to infestation with larvae of a nematode genus *Trichinella*. This disease is acquired by ingestion of raw or inadequately cooked meat-products containing encapsulated larvae. These larvae are released after gastric digestion and mature into adult worms that penetrate the mucosa of the intestine (De Bruyne et al., 2006b). After fertilization, the female sheds new larvae which disseminate throughout the host to find their definitive location, the striated muscle where they encysted. This larval migration may result in severe lesions, particularly when larvae migrate in the heart or in the brain (Dupouy-Camet, 2000).

The hosts implicated in the life cycle of *Trichinella* are carnivores and scavenger animals. The domestic cycle can implicate breeding pigs and Human, and in some cases the dog which can be contaminated especially when eating infested meat of swine. The sylvatic cycle implicates wild boars (De Bruyne et al., 2006a), wild carnivorous (Blaga et al., 2007), as red foxes, and rodents. There is a link between domestic and sylvatic cycles when eating contacts happen between wild and domestic animals.

CORSICA AND TRICHINELLOSIS

Until 2004, the island of Corsica was considered as a '*Trichinella*-free' area: no human or animal infection had been reported. Until this year, some hunting surveys on wild boars showed an increasing of the visible seropositivity but no parasite had been found in wildlife in general (Table 1).

In 2004 a swine outbreak is discovered in the official slaughterhouse of Bastelica (middle of the Southern Corsica): 10 pigs coming from a farm of the valley of the Taravu (Fig. 1) are detected positives with the standard method (artificial digestion); in each of these pigs larvae of *Trichinella* *britovi* are isolated, with a parasitic charge between 0.5 and 144 lpg (larvae per gram) ((Vallée et al., 2004)). An epidemiologic survey in domestic pigs from farms around the outbreak revealed no additional positive samples. During the following hunting campaign wildlife samples were analysed and remained negative excepted for one red fox, a female killed at 5km of the area of the positive pigs farming. This fox was infested with *Trichinella britovi* (Tab. 1 and fig. 1), with a parasitic charge of 1 lpg.

To explain the origin of the Corsican *Trichinella* emergence, two hypothesis were proposed: (i) the introduction, many years ago, of a continental infested animal (a dog or a wild boar brought on the island) which died on the area of feeding of the pigs, or (ii) the discovery of an enzootic infestation of very low prevalence, not bringing it to light before. Considering this last hypothesis, fires of 2003, which occurred at 7km from the outbreak, may have lead to exceptional movement of wild animals and revealed the presence of the parasite. In this case it is more difficult to explain why larvae have been detected in pigs and fox but not in wild boars.

Nevertheless, beyond this not elucidated question of the outbreak's origin, 3 others questions are still on: (i) what is the present status of the island? , (ii) have the parasite spread to others micro-regions of Corsica? , (iii) what is the risk of Trichinellosis for the public health in Corsica?

PIG BREEDING AND WILDLIFE IN CORSICA

With an official swine population's size of 26360 pigs (data from the 2001's agricultural census report), the pig breeding in Corsica is exclusively based on an extensive farming system: free ranging pigs living in a forest and mountainous pastoral system. In this context of outdoor system and in application of European Community rule 2075/2005 - article 2, each pig has to be officially slaughtered with a systematic meat inspection (standard method). But because of a strong tradition of on-farm slaughtering, and farms sometimes far from the slaughterhouses, the official slaughtering still represents less than half of the total: in 2006-2007 for example 4576 pigs have been slaughtered in one of the 4 slaughterhouses (veterinary inspection data), for an estimated number of pigs slaughtered per year in Corsica of 12000. Geographically speaking, some micro-regions have still a low proportion of pigs officially slaughtered, as the valley of the Taravu (area of the breakdown in 2004) or the region of 'Deux Sorru' (middle west). This situation leads to one conclusion in particular: the sanitary surveillance via slaughterhouses gives only a partial picture of the reality.

About wildlife, Corsica is a region of important and traditional hunting of wild boars. This hunting is not well structured: there is no hunting plan and many areas are still not organised in hunting society or association. Furthermore poaching is per definition a non-official practice but well-known for being still real in Corsica. Consequently to estimate accurately the population size of wild boars remains difficult too. Official data from the departmental hunting federations report an average of 20000 wild boars hunted each year. Locally the densities of wild boars can be high, especially because of the crossing between domestic pigs and wild boars with creation of hybrid animals, more prolific. No study reports exact data on vulpine population but densities of red foxes is known to be also high. Several tales of breeders report furthermore predation on piglets.

These description of the wildlife and breeding context help to understand the important interactions between free-ranging pigs and some wild animals, which can be potentially hosts for Trichinella as foxes and wild boars. All the more so that, if game meat is eaten in familial and friendly circle wild boar meat especially is also consumed in restaurant, with no sanitary inspection.

ECO-EPIDEMIOLOGICAL APPROACH: METHOD AND FIRST RESULTS

As seen above, the key points of the regional situation are: slaughterhouses and partial representation of the sanitary situation, large interactions between pigs and wildlife, importance of the hunting of wild boars and no sanitary inspection of game meat. All these particularities of the Corsican context have led an approach to focus on the epidemiological study of wild reservoirs: (i) to detect the infestation in wild boars in order to assess the risk for public health, (ii) to estimate the prevalence of the infestation in red fox's population as a sentinel of the parasite on the island.

Sample sizes have classically been calculated in function of both attempt prevalence and estimated population sizes. In order to detect the presence of *Trichinella* in the wild boars' population with a detection level of 0.1% with a confidence of 95%, an aim of 1 thousand samples per department, so 2 thousands for the entire island, has been definite. For red foxes, with an attempt prevalence of about 5% (Van der Giesven *et al.*, 1998; Criado-Fornelio *et al.*, 2000), 200 samples are expected.

The disposal of samples' collection is based on 4 stages: (i) mobilisation of voluntary hunters and explanation to them concerning the sampling method (ii) transport of the samples until the laboratories, (iiI) analysis, (iv) data centralisation and restitution of the results. For wild boars, during all the hunting season, from the 15th of August to the 15th of January, hunters have sampled the red muscle part of the diaphragm. For red foxes, hunters and agreed trappers were told to send the entire specimen to the laboratories. There the leg muscles were sampled.

The transport to one of the 2 Departmental Veterinary Laboratories (LVD) of the wild boars' samples or foxes' body was operated with the help of a net of intermediaries: Federal Hunting guards, veterinarians, agents of the National Institute for the agricultural Research (INRA). At the LVD, analyses were performed using artificial digestion method in pool on, respectively, 5 g per wild boar's sample or 20 g per fox's sample. The protocol plan also to analyse all the samples by serology on muscle fluid, to compare data from direct and indirect methods; this analyse will be performed at the French Food Safety Agency in a second time. The fourth stage of the disposal is a data centralisation by the coordinator at the INRA and a restitution of the results to the hunters, individually, each month, and collectively at the end of the hunting season, in collaboration with the local institution for veterinary inspection.

At the end of a first season of sampling (Table 2), 670 samples of wild boars have been collected; all were negatives with the direct method. Considering the geographical repartition of the sampling effort (fig. 2a), some areas appear not at all or not well represented. So a second season of sampling was necessary to complete the sample size and cover the entire island, especially the region of the outbreak. Only 32 red foxes were collected (Table 2 and fig 2b) and were negatives with the direct method. Because of the great interest of the fox as sentinel, we have to try to overcome the real difficulty to obtain samples (no tradition of hunting or official trapping) and conduct a second season of sampling.

CONCLUSIONS

In a context where the low proportion of pigs sacrificed in an official slaughterhouse poses the question of the sanitary image of the pig breeding in Corsica, investigation on the wild hosts is of a great interest. But one of the local difficulties is to estimate more precisely the population sizes, which is one of the aims of the research. In the same time, when studying the risk for human health, one of the points to consider is the survival of the Corsican strain against freeze and traditional salting. This is the objective of some works presently conducted in collaboration with the French Food Safety Agency. Overall, in an aim of a construction of a real network of zoonosis' surveillance in Corsica, this organisation around Trichinellosis' surveillance is a first step. But the main difficulty is to keep the actors mobilised.

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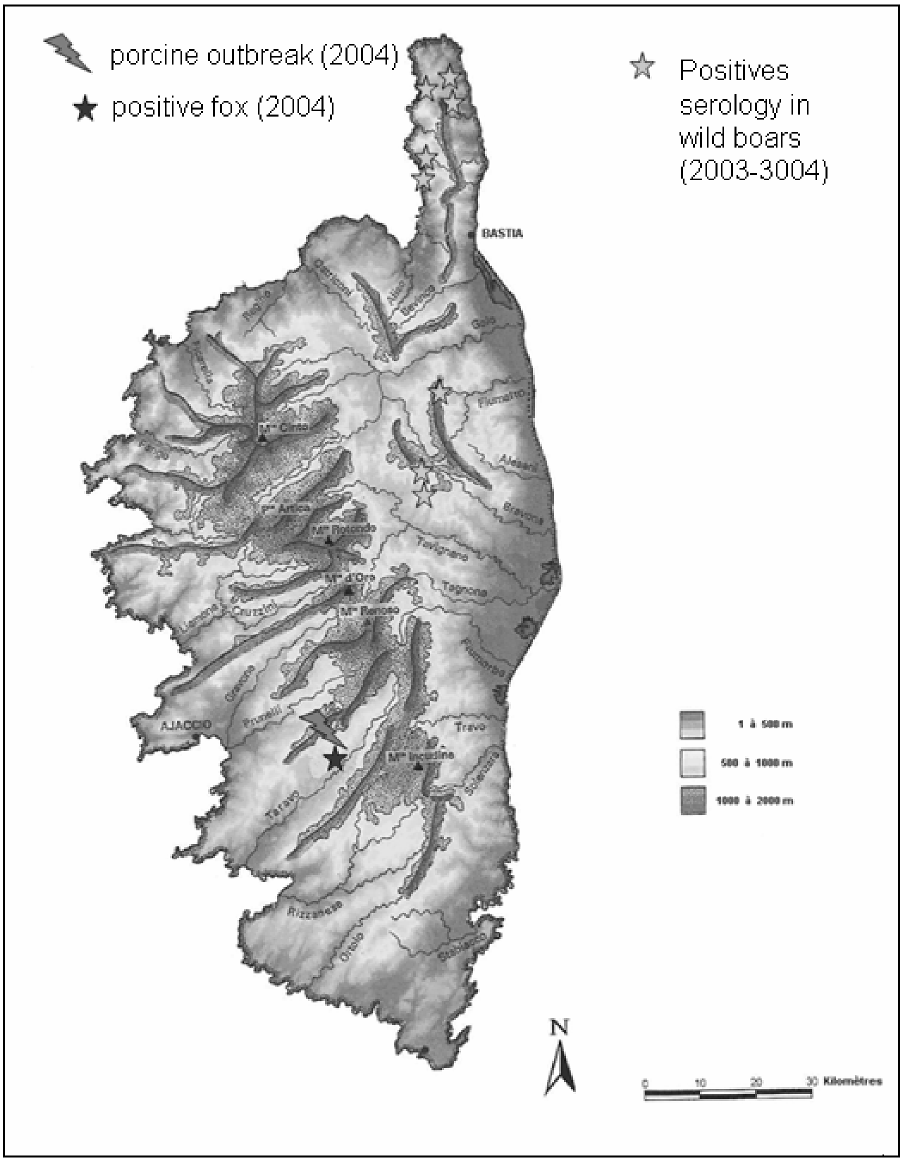

Figure 1. Cartography of the cases of positives serology or peptic digestions in Corsica before the present study, referring to the table 1.

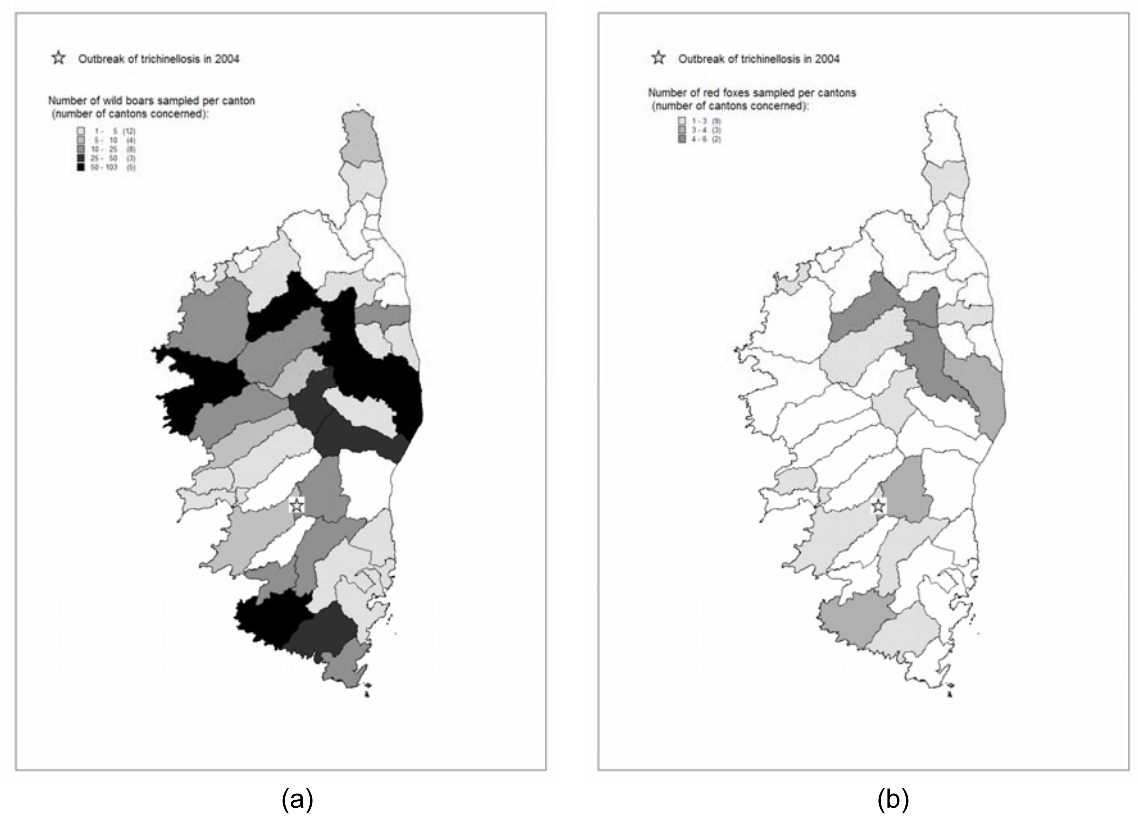

Figure 2. Geographic repartition of the samples collected during the hunting season 2006-2007 in Corsica : (a) wild boars, (b) red foxes.

Table 1. Compilation of the existing data on Trichinellosis in the region of Corsica before the present study.

Years	Species	Area	Method	Results
2000-2001	Wild boars	All Corsica	serology	1**+**/ 110
2003-2004	Wild boars	North Corsica	serology	8**+**/ 97
2004	Breeding pigs	Valley of the Taravu	pepsic digestion	10**+**
2004	Red foxes	Valley of the Taravu	pepsic digestion	1**+**/ 14
2004-2005	Wild boars	All Corsica	pepsic digestion	0**+**/ 387

Table 2. Results of the surveillance of Trichinellosis in the populations of wild boars and red foxes of Corsica during the hunting campaign 2006-2007, using the peptic digestion (for wild boars: diaphragm muscle, 5g per sample, pool of 20 samples ; for foxes: leg muscle, 20g per sample, pool of 5 samples). All the samples analysed during this first campaign are negatives in direct research of larvae.

Area	**Wild boars**		**Red foxes**
	sampled	**analysed***	
North Corsica	392	391	22
South Corsica	273	266	10
Total	665	**657**	**32**

* Some samples have not been analysed (not enough quantity of muscle)

ISOSPORIASIS IN DOMESTIC PIGS OF SARDINIA (ITALY)

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SUMMARY - The aim of the present study is to evaluate the presence and the management of Isosporiasis by *Isospora suis,* in swine farms of Sardinia. Copromicroscopical investigations carried out on 55 breedings (34 intensive, 21 extensive) have shown the presence of oocysts of *I. suis* in 3.6% of the farms. Isosporiasis affected almost exclusively suckling piglets from 5.9% of intensive examinated breedings. The evaluation of the efficacy of the chemioprophylactic treatment with Toltrazuril (20 mg/kg) made in a positive farm at 3th day of life of piglets show statistically significative results (growth rates) compared with control farms.

Key words: *Isospora suis*; epidemiology; toltrazurile; Sardinia

INTRODUCTION

Among the parasitic noxae that can interfere negatively on the industrial pig farming, Isosporiasis is frequent increasingly refereed. It is caused by *Isospora suis*, apicomplexan protozoa responsible of diarrheal forms in piglets, that could be cause significant losses in the infected farms (Roepstorff *et al.*, 1998; Torres *et al.*, 2004).

The infected piglets eliminated oocysts in the environment that sporulates rapidly, forming within them two sporocysts each containing four sporozoites, that represent the exogenous phase of the biological cycle of the parasite. The coccidiosis in piglets under sow is a disease reported in many countries around the world (Meyer *et al.*, 1999; Cotrell, 1998; Chae *et al.*, 1998; Sotiraki *et al.*, 1998; Larsen, 1996; Otten *et al*., 1996; Lindsay and Blagburn, 1994).

Recent surveys conducted in northern Europe, United States and Australia have shown that *I. suis* is the most frequently parasite found in piglets between 7 to 14 days of age and that the presence of infection is associated with diarrhea in more than 50% of infected animals (Driessen *et al.*, 1993; Meyer *et al.*, 1999)

Currently against Isosporiasis is suggested and, and in many cases carried out a prophylactic treatment with toltrazuril, triazinon derivative anticoccidial drug, which significantly reduces the elimination of oocysts and and diarrhea symptoms in infected piglets (Gualdi *et al*., 2003; Busse, 2004).

In this paper were reported the results of an investigation to determine the presence of Isospora infection in pig breedings of Sardinia and the results of two chemioprophylactic protocols applied in intensive production pig farms.

MATERIAL AND METHODS

During 2005 and the first half of 2006 coprological investigations on samples from piglets maximum of 1 month of life of 55 breedings (34 intensive and 21 free/wild) of Sardinia (pools of at least two groups of piglets per breeding) were conducted aimed to find oocysts of *Isospora suis* through the Ridley methodology (Martineau *et al*., 1994).

The test of the performances of chemioprophylactic protocols was carried out at a pig intensive breeding in south-central Sardinia (about 1,200 sows), in which had been previously detected the presence of oocysts *I. suis* in piglets faeces. Since the day of birth 31 piglets litters reared in cages with grilled have been monitored. For each of these litters at day 7, 21 and 28 after the birth, faecal samples were collected and then put togheter to form a single pool for litter represented by no less than four different faecal masses.

The coprological analysis aimed to find oocysts *I. suis* were performed according to Ridley methodology (Martineau *et al*., 1994). The sediment obtained using the described above method was also used for the quantitative research of oocysts (opg) through McMaster slide.

Recovered oocysts, along with those obtained from coprocolture prepared in 2% potassium dichromate were classified using the identifications keys reported by Pellerdy (1974), Boch and Supperer (1980) and Euzeby (1987).

The piglets litters were random divided into three groups: group A, n ° 8 litters whose members were treated at 3 days after birth with a oral dose of 20 mg/kg of toltrazuril suspension equal to 0.4 ml suspension of 5% Baycox ® (Bayer); group B, n ° 8 litters, that, at 3rd, 4th and 5th day after birth, were treated with a Sulfamethazine sodium and trimethoptrin solution respectively of 27 and 5 grams in 100 ml of water for injections, at a dose of 2 ml per head. Group C, of n. 15 litters were used as control. All of the subjects of the litters were weighed the day of birth (1st day) and then at day 7 and 28, for calculating their daily increase weight. From the day of birth in all the litters of the groups A and B and in the first 8 litters of Group C was noted the consistency of the faeces (normal or diarrheal).

RESULTS AND DISCUSSION

Coprological investigations allowed to detect the presence of *I. suis* oocysts in 3.6% of the farms controlled. Particulary, the parasitosis affected only piglets under sow in 5.9% of the monitored breedings.

The effectiveness test of anthelmintic treatments showed a significantly lower level of the elimination of oocysts at day 21 in subjects of the litters treated with toltrazuril (group A) than control animals (group C) (Mann-Whitney test - P = 0.021). A significant reduction of infection prevalences has been registered at day 21 in the Toltrazuril treated litters (group A), who were all negative to parasites, and control (group C) (χ^2= 10.37; P = 0.001) and between toltrazuril treated litters and those treated with sulfamethazine-trimethoptrin solution (group B) (χ^2= 4.65; P = 0.031).

The weight monitoring of each piglet has showed a significant increase in the analysis of variance at day 28 (F = 3.883; P = 0.032). Weight increases showed significant changes at day 7 (F = 3.805; P = 0.035), when the subjects treated with sulfamethazine-trimethoptrin recorded higher values. These differences were bigger at day 28 (F = 5.871; P = 0.007), when increased weight significantly higher values were found in subjects treated with toltrazuril compared with the animals of the other two groups.

Diarrheal faeces throughout the period of observation were significantly lower in subjects of group A (36 diarrheal stool samples in 28 days - average 4.5-monitoring samples/litter) than in group B (79 samples in 28 days average 9.9-monitoring samples/litter) and group C (85 samples diarrheal stools in 28 days of monitoring - average 10.6 samples/litter).

The trend of the number of diarrheal stool samples showed a strong increase from 12 to 18 day, when were noted an increase of these samples in Group C. A further increase in the emission of these samples was observed in Group B from 22 day onwards.

The epidemiological investigation has detected Isosporiasis only in few intensive breedings (5.9%). This data is significantly lower than what found in a survey previuosly done in Sardinia using toltrazuril, where were found 85,2% positive farms. It seems that the routinely implementation of this treatment applied in almost all intensive farms in the territory may have resulted in a marked reduction of Isosporiasis in the past years. The effectiveness test of the two chemioprophylactic protocols allowed today to highlight the usefulness of a toltrazuril treatment against in under sow piglets in intensive farms, as already reported previously in similar experiences (Rypula and Porowski, 2004; Mavromatis et al. , 2004).

This investigation also revealed that the toltrazuril molecule administered at the third day of life of piglets will be able to significantly reduce the number of infected subjects, with a significative reduction of the number of oocysts eliminated and of the reduction of environmental contamination. Needless for Isosporiasis control purposes was in this work the sulfamethazine-trimethoptrin protocol. It is clear therefore that unfortunately coccidiosis by *I. suis* could play an important pathogen role in intensive farms, if not monitored with usuful and effective prophylactic plans.

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CYSTIC ECHINOCOCCOSIS IN PIGS AND WILD BOARS OF SARDINIA (ITALY)

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SUMMARY - The aim of the present study is to evaluate the presence of Cystic Echinococcosis in pigs and wild boars of Sardinia. The survey was carried out on pigs slaughtered in familiar butcheries during the years 2005-2007, while data and hydatid material from wild boars were recovered from shouted animals during the hunting campaign from 2004 to 2006. A total of 342 pigs and 461 wild boars were observed and hydatid disease was revealed respectively in 11,1% and 3,7% of the animals. Fertility was of 7,6% in pigs (68,4% of the positives animals harboured viable cysts). Thirty-nine hydatids isolates from pigs (36) and wild boar (3), were then strain typed after DNA extraction and sequencing of COI and ND partial genes. The common sheep strain was isolated in 37 pigs while the G7 or pig strain was observed in two swine. The wild boar samples harbour all to the G1 strain.

Key words: Echinococcus granulosus, Sardinia, strain typing, G7, Pig, Wild boar

INTRODUCTION

Cystic Echinococcosis (CE) in pigs in Sardinia is a parasitic disease not easy indagabile for some rearing problems and also for the consequences linked to other animal health problems of this species, primarily the Swine Fever, widespread the territory despite many controls projects carried out in the past, and since 2005 also by the spreading of some *Trichinellosis* cases.

The pig breeding in Sardinia presents a heterogeneous reality which recognizes a major component based on intensive breeding, on a family breeding (usually few animals), and another kind of breeding, based on illegal rearing of pigs free/wild in the fields.

Beside these realities Sardinia also has a large population of wild boar, which often live in promiscuity with pigs "domestic", and whose consistency in some districts makes it difficult to manage health aspect. In this context it seemed useful to report preliminary results (especially as regards the wild boar) of an investigation to clarify some important epidemiological and biomolecular aspects of CE in Sardinia, important zoonoses historically present in the island in order to contribute to define better the role of these ungulates in the trasmission dynamics of the infection.

MATERIAL AND METHODS

Starting from March 2005 until January 2007, a total of 2,330 pigs from 15 intensive breedings and 342 pigs from familiar butcheries were examined. Through the collaboration of field veterinaries and hunters, we also collected data on 461 wild boar killed during hunting seasons held in several municipalities of Sardinia between 2004 and 2007 (November-January period).

On every pig slaughtered found positive to CE infection, the number, location and type of hydatid were evaluated according to the following classification: acephalocysts or fertile. Fertility was assessed by microscopic observation of the germinal layer. The viability of protoscoleces was determined by examination at 10X without staining, observing flame cells movements. From each cyst, laminar layers and protoscoleces were removed and stored at -20°C. DNA was extracted from 39 samples of hydatid material obtained from pigs (36) and wild boar (3) using a commercial kit (Roche DNA template extraction kit). The protocol established by Dinkel *et al.* (2004) was performed on all DNA samples for a first screening of all isolates in order to discriminate the G1 strain of *E.*

granulosus from the G5 and G6/7 strains with four different PCR reactions. Polymerase chain reaction (PCR) was carried out for the amplification of target sequence of the mitochondrial 12S rRNA gene in a 25µl volume containing 10 mM Tris–HCl (pH 8,3), 50 mM KCl, 2 mM $MgCl_2$, 250µM of each dNTP, 2U of *Taq* polymerase (Roche) and 25 pmol of each primer (Dinkel *et al.*, 2004). Amplification was carried out for 40 cycles as follows: denaturation for 30 s at 94 °C, annealing for 1 min at 57 °C and elongation for 40 s at 72 °C. After amplification, 10 µl of the amplification products were detected and photographed on a 1.5% ethidium bromide stained agarose gel. Then sequencing reactions there were undertaken on PCR products as described by Bowles and McManus (1993a; 1993b) for NADH and COI mitochondrial genes. Genomic DNA was isolated as described previously and mitochondrial DNA regions were amplified from 10–20ng of template by PCR. ND1 (~500bp) was amplified with primers JB11 and JB12, while COI with primers JB3 and JB4.5 (Bowles and McManus, 1993a; 1993b; Bowles *et al.* 1992). Polymerase chain reactions (25µl) were performed in 10mM Tris–HCl (pH8.4); 50mM KCl; 2mM $MgCl_2$; 250µM of each dNTP; 25pmol of each primer and 2 U *Taq* polymerase (Roche) under the following cycling conditions: 94°C, 5min (initial denaturation), followed by 30 cycles of 94°C, 30s (denaturation); 55°C, 30s (annealing); 72°C, 30s (extension), followed 72°C for 5min (final extension). Thirty-five cycles of denaturation (94 C for 30s), annealing (55 C for 30s) and extension (72°C for 30s) were carried out. For each set of PCR reactions, negative controls (no DNA) were included. DNA from PCR products was purified with a commercial kit (MinElute™ PCR Purification Kit, Qiagen S.A.). Sequencing was performed on the ABI Prism 310 (Applied Biosystems) with the ABI Prism Big Dye Terminator Kit (Applied Biosystems) using the corresponding PCR primers. Nucleotide sequence analysis was undertaken using the National Center for Biotechnology Information BLAST programs and databases. Multiple sequence alignments were made with the ClustalW method with Bioedit software and compared with GenBank sequences.

RESULTS AND DISCUSSION

No cases of CE were highlighted in pigs reared intensively. A prevalence of 11.1% (38) in pigs home slaughtered was detected, in which was highlighted a fertility of 7.6% equal to 68.4% of positive subjects (26). There was no significant difference between infection prevalences found in the liver and lung ($\chi 2$= 0,5; P= 0,478).

In pigs from familiar butcheries massive infection (> 10 hydatids) was detected in two pigs with cysts localized also in myocardial, spleen, and skeletal muscles. In boars instead was detected a prevalence of 3.7% (17). Of the 39 DNA isolates subjected to sequencing, 36 pigs and 3 wild boars, the 5.1% (2) belonged to G7 or Pig strain, while 94.9% (37) belonged to G1 or sheep strain (see table 2). All isolates from wild boars belonged to strain G1. The sequences obtained were registered in NCBI network (Accession numbers: DQ023703; DQ062857; DQ062858).

The investigation has confirmed the CE problem of pigs and wild boar in Sardinia even if the prevalence rates and the percentage of subjects with fertile cysts compared with the recent past, show a clear reduction of the trend (Garippa, pers. comm.). However, according to the data obtained, pigs could be a dangerous source of infection for the persistence of this zoonoses in the island.

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A REVIEW OF PARASITES FOUND IN THE SICILIAN BLACK PIG

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SUMMARY - In this paper, the results of parasitological surveys carried out on a total of 72 Sicilian Black Pigs (SBPs) raised in two different breeding systems in Sicily are summarized. Parasitological results and epidemiological data showed significant differences in parasite species richness and in the frequencies of infestation between pigs bred in extensive and indoor-housing systems. The low sanitary standards of extensive breeding systems are highlighted by the high frequencies of muscular cysts of *Sarcocystis* spp. (85%) and by the dangerous presence of hydatidid cyst (49%) in the liver and lungs of the sampled pigs. All the SBPs examined in our surveys were *Trichinella*-free. Review results support the necessity of improving sanitary and hygienic levels of the Sicilian extensive breeding systems in order to reach higher standards for animal health as well as for the quality of products.

Key words: Sicilian Black Pig, parasites, epidemiology, breeding systems, Sicily.

INTRODUCTION

The Sicilian Black Pig (SBP) is a breed-population of 1384 heads raised mainly in the Nebrodi Regional Park area (Liotta, 2006). However, the SBP production system is mainly based on extensive systems; in the last 10 years efforts have been made to improve the production system by moving traditional breeding systems to plein-air rearing with alimentary integration. Surveys on parasite infestation can be used to assess the health status of an animal population and to make inferences on the hygienic level of the farming system. Therefore, the aims of this review are to summarize our survey results on parasites and parasitic diseases found in the SBP breed in two different breeding systems in Sicily supporting the necessity of improving the sanitary and hygienic levels of the breeding system in extensive conditions.

MATERIAL AND METHODS

A total of 72 SBPs, 41 from free-ranging the extensive system and 31 from the indoor-housing system aged between 1-3 years were randomly sampled and examined for a parasite presence after slaughter. The digestive tract was isolated and inspected for parasites by the Total Worm Count technique (Maff, 1986). Lungs, liver and spleen were isolated and checked for a parasite presence by palpation and visual examination whereas portions of the heart and diaphragm were digested using a peptic chloride solution and then observed under the microscope for the presence of protozoa cysts (Jacobs, 1960). The parasites found were identified using usual taxonomic keys (Soulsby, 1986; Anderson, 2000) and a descriptive statistic was calculated for each parasite species found (Margolis *et al*., 1982). The difference in frequencies of parasitosis, according to the type of breeding system, was analyzed using the chi-square test (Hawkins, 2005).

RESULTS

In table 1, parasitic species/diseases found and their epidemiological indices ranked by the anatomical area and the type of breeding system are reported. Eleven parasitic species were identified in all; parasitic fauna richness and the epidemiological indices were significantly higher in

SBPs raised in the extensive system than in those from the indoor-housing system. Among the helminths of the digestive tract a higher prevalence was recorded for *Ascarops strongylina* (76%), *Globocephalus urosubulatus* (76%) and *Oesophagostomum dentatum* (90%) isolated from the stomach, small and large intestines, respectively. Infestations sustained by *Ascaris suum*, *G. urosubulatus*, *Macracanthorhynchus hirudinaceus* and *Phisocephalus sexalatus* were observed only in the SBP bred in the extensive system. The infection by *Trichuris suis* was present only in the SBPs sampled from the indoor-housing breeding. *Metastrongylus elongatus* and *M. pudendotectus* were the species found in the lung area; nevertheless, the former species was absent in pigs from the indoor-housing system. Liver cystic echinococcosis was observed in 49% of the SBPs from the extensive system. A presence of muscular cysts of *Sarcocystis* spp. was recorded in 85.4% and in 13% of the SBPs raised in extensive and indoor-housing systems, respectively.

DISCUSSION

All the parasitic diseases reported in this review are typical of swine. Some of these such as infestation sustained by *A. suum*, *A. strongylina*, *O. dentatum*, *M. hirudinaceus* and *Metastrongylus* spp. have important negative effects on animal health as well as on animal productions. Others such as cystic echinoccocosis and sarcocystosis have also important Public Health relevancies. Moreover, the sharp differences in the parasitic fauna richness and the frequencies of infestation found between the two breeding systems analyzed highlight the sanitary weakness of the extensive rearing system. These findings suggest that even if animal-friendly production systems like extensive or plein-air fit the regulations well for animal welfare and organic production, it is necessary to improve the sanitary and hygienic levels of these breeding systems to reach higher standards for animal health as well as for the quality of products.

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Table 1. Comparison of parasitic species/diseases and epidemiological indices between SBPs raised in the extensive and indoor-housing systems.

		Extensive (n = 41)			Indoor housing (n = 31)		
Area	**Parasite species / Disease**	**P (%)**	**Im**	**Am**	**P (%)**	**Im**	**Am**
stomach	*Ascarops strongylina*	76*	45	34	3.2*	20	0.6
stomach/small intestine	*Phisocephalus sexalatus*	5	2	0.02	-	-	-
small intestine	*Globocephalus urosubulatus*	76	80.5	61	-	-	-
	Macracanthorhynchus hirudinaceus	32	2	0.6	-	-	-
	Ascaris suum	46.3	5	2.3	-	-	-
large intestine	*Oesophagostomum dentatum*	90*	129.5	117	3.2*	66	2
	Trichuris suis	-	-	-	10	4.3	0.4
lungs	*Metastrongylus elongatus*	48.4	22.4	9	-	-	-
	Metastrongylus pudendotectus	29.2*	25	8	6.4*	7.5	0.5
liver / lungs / spleen	Hydatidosis	49	-	-	-	-	-
heart / diaphragm	*Sarcocystis* spp.	85.4*	-	-	13*	-	-

Table legend: P (%) = prevalence; Im = means intensity of parasites in infected animals; Am = means intensity of parasites in sampled animals.
* frequencies analyzed by the chi-square test are significantly different ($p < 0.01$)

ECTOPARASITOSES IN PIGS SLAUGHTERED IN SICILY: PREVALENCE AND DIAGNOSTIC TOOLS

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SUMMARY - Study aim was to investigate the prevalence of ectoparasitoses in pigs slaughtered in Sicily, by different diagnostic tools. Samples were collected from 500 pigs randomly chosen at the time of slaughtering. The presence of specific serum antibodies against *S. scabiei* was detected in 326 (65.2%) pigs by the ELISA test. However, *S. scabiei* was found only in 11 samples (2%) and *Haematopinus suis* in 55 (11%) by the ear scraping. Dermatitis Score evaluation produced a mean value of 0.25.

Key words: *Sarcoptes scabiei* var. *suis*, *Haematopinus suis*, pig, ELISA, dermatitis score, diagnosis, Sicily.

INTRODUCTION

Louse infestation by *Haematopinus suis* (Fig.1) and mange by *Sarcoptes scabiei* var. *suis* (Fig.2) are the main ectoparasitic diseases of swine. *H. suis* infestation is cause of nuisance and skin lesions due to the intense pruritus; *S. scabiei* var. *suis* infection is cause of chronic allergic dermatitis in both breeding and fattening pigs. Both parasitoses are causes of important economic loss in pig industry. A recent review on the current status of livestock ectoparasitoses in Europe reported that sarcoptic mange is still common in pig breeding and fattening farms with prevalences ranging from 8.3% to 55.8% (Colebrook and Wall, 2004). In Italy, sarcoptic mange was present from 5.7% to 62% of the inspected pigs (Griglio *et al*., 1996; Martelli *et al*., 1998; Galuppi *et al*., 2006). On the other hand, few are the reports on *H. suis* infestation in European pig industries. Damriyasa *et al*. (2004) in Germany, reported the presence of *H. suis* in 68 (2.5%) of the 2754 examined sows. In Italy, the only data to our knowledge are brought back from Poglayen *et al.* (2001) that reported a prevalence of 7% for *H. suis* in the Sicilian Black Pigs bred in a free ranging style.

The aim of this paper was to run a descriptive study on the prevalence of the ectoparasites in pigs slaughtered in Sicily by direct (i.e. ear scraping) and indirect (i.e. Dermatitis Score (DS) and ELISA) diagnostic methods.

MATERIAL AND METHODS

The survey was performed on 500 light-weight pigs slaughtered in Sicily (266 in Messina, 126 in Catania and 108 in Enna provinces). Pigs were chosen by a systematic way of sampling performed from November 2006 to December 2007 in different slaughterhouses and in different days of the week. From each carcass sampled one ear and external auditory canal were removed. The ears were inspected for *H. suis* presence and then the skin inside of the ears was carefully scraped and checked by microscope for mites presence after digestion in KOH 20% solution. Digested material was also processed by flotation technique in solution of 1300 s.g. and observed at microscope for mites identification. The presence of skin lesions (erythematous papular dermatitis) was evaluated in each carcass sampled for DS in scale ranging from 0 to 3 according to the severity of the lesions as reproduced in Fig.3. Blood was also collected at the time of slaughtering and the sera tested for the presence of specific serum antibodies against *S. scabiei* var. *suis* (IgG) by ELISA test (Smets *et al*., 1998). Results of the ELISA test were evaluated by Multiscan RC Labsystem photometer and expressed as Correct Optical Density (COD) using the formula proposed by Lowenstein *et al*., 2004. COD ≥ 0.4 will be considerated indicative of the presence of specific antibodies against *S. scabiei* var. *suis* .

Statistical analysis were performed by chi-square test for the differences in frequencies, Student's t test for the differences in data series, whereas, correlation between DS and COD values was analyzed by Pearson correlation test.

RESULTS

In table 1 are summarized results of tests ranked by provinces of sampling. By the ear scraping method *H. suis* was isolated in 55 ears (11%), *S. scabiei* was observed in 11 samples (2%) whereas mites of *Demodex* sp. were present only in one sample. Mixed infections sustained by mites and louses were never observed. The flotation technique showed the higher sensibility than digestion procedure. By the ELISA test specific antibodies against *S. scabiei* were detected in 326 samples (65.2%) with COD values equal or higher than 0.4. Erythematous papular dermatitis was observed in few carcasses and the overall DS was 0.25. *S. scabiei* ear scraping positive samples showed significant higher values of DS ($p = 0.04$) and of COD ($p < 0.001$). DS and COD values were not correlated ($r = 0.06$).

DISCUSSION

Our results confirm that ectoparasites are still a health problem in pig industry. The ELISA test results suggest a wide circulation of sarcoptic mange in pigs slaughtered in Sicily. However, it is important to note that more than 60% of these pigs came from other European countries, so our results reflect a problem not only restricted to the Italian pig industry. A sharp difference was observed between the results of the ear scraping and the ELISA test. This difference could be due to treatments before slaughtering that remove mites but leave an active residual immunity up to 6-12 months that is still valuable by the serological tests. However DS values were significant higher in *S. scabiei* positive samples, these were ever below scores indicatives of erythematous papular dermatitis. Moreover, in our survey the DS values were unrelated to the COD values. Thus, DS seems to be a weak method for sarcoptic mange diagnosis while ELISA should be preferred as screening test in epidemiological surveys.

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Table 1. Number of samples and results of ear scraping, Average Dermatitis Scores (ADS) and Correct Optical Density (COD) divided by province of sampling

		ES		ADS			COD	
Province	N	+ *S. scabiei* (%)	+ *H. Suis* (%)	Samples	+ *S. scabiei* (± SD)	+ *H. suis* (± SD)	Samples	+ *S. scabiei* (± SD)
Messina	266	4 (1.5)	29 (5.1)	0.2	0.6 (± 0.3)	0.2	0.7	1.4 (± 0.8)
Catania	126	4 (3.2)	4 (3.2)	0.2	0.4 (± 0.3)	0.5	0.5	0.8 (± 0.8)
Enna	108	3 (2.8)	22 (20.4)	0.4	0.5 (± 0)	0.3	0.8	2 (± 0.8)
Total	500	11 (2.2)	55 (11)	0.2*	0.5*(± 0.2)	0.3*(±0.3)	0.7*	1.4*(± 0.8)

* Mean value

Figure. 1. *Haematopinus suis:* dorsal view.

Figure. 2. *Sarcoptes scabiei:* ventral view.

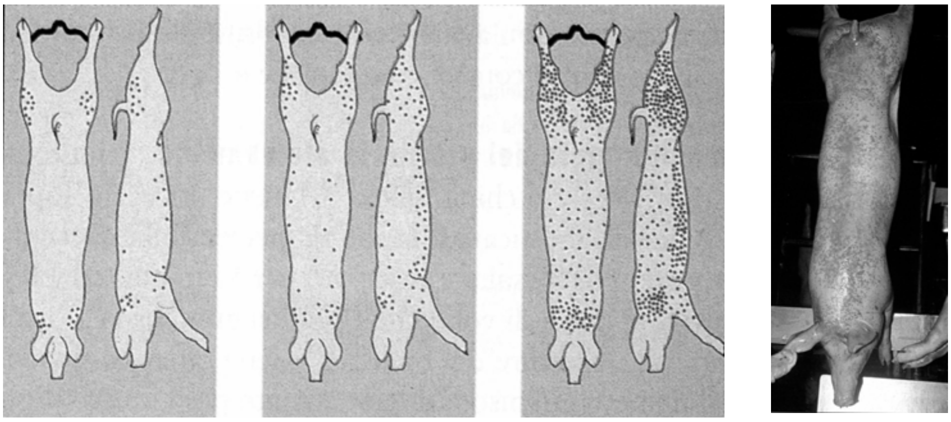

Figure. 3. Examples of Dermatitis Score. Modified from *Genchi & Kramer* (2000).

SESSION 3
FEEDING AND REARING SYSTEMS

EFFECTS OF FEEDING AND REARING SYSTEMS ON GROWTH, CARCASS TRAITS AND MEAT QUALITY IN PIGS

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SUMMARY - Animal growth performance and quality of pork depend on the interactive effects of genotype, rearing conditions, pre-slaughter handling, and carcass and meat processing. This paper focuses on the effects of feeding and rearing systems on growth performance, carcass composition, and eating and technological qualities of pork. The feeding level and pattern (restriction / realimentation) and the composition of the diet (protein level, protein to energy ratio, fatty acids composition) can be used to manipulate growth rate and composition of weight gain at both carcass and muscle levels, and thereby modify carcass and meat quality. The effects of indoor housing conditions (floor type, space allowance, ambient temperature), alternative rearing with outdoor area, or free-range (extensive) systems on animal performance, carcass and meat quality are presented, with a special focus on the Mediterranean production systems, in which interactions between genotype (local breeds) and extensive finishing conditions lead to high eating quality of pork and pork products.

Key words : Pigs, Feeding, Rearing conditions, Performance, Meat Quality

INTRODUCTION

Growth performance of pigs, carcass composition and quality of pork and pork products depend on multiple interactive effects of genotype (genetic background, presence of major genes hal and RN^{-}), rearing conditions (feeding level, housing and environmental conditions, production system), pre-slaughter handling, and carcass and meat processing (reviews of Sellier, 1998; Monin, 2003; Rosenvold and Andersen, 2003; Terlouw, 2005). This paper focuses on the influences of feeding and rearing system on growth performance, carcass and muscle composition, and eating and technological qualities of pork.

The effects of feeding level, composition (protein:energy ratio) and pattern (restriction-realimentation) as tools to manipulate growth rate, composition of weight gain, intramuscular fat (IMF) deposition which is often associated with improved meat sensory traits (DeVol *et al.,* 1988; Fernandez *et al.,* 1999; Wood *et al.,* 2004) and thereby improved pork quality, are described. Variations in muscle lipid composition and nutritional value of meat through dietary supplementation (fatty acids, antioxidants) are also presented.

Considering pig rearing systems, the specific effects of housing conditions (ambient temperature, floor type, space allowance…), and outdoor and free range rearing systems on animal performance and carcass and meat quality are described. Finally, a special focus is given on specific production systems from the Mediterranean area, involving local breeds that are extensively pastured and slaughtered at advanced age for the production of high-value dry-cured products.

Except for the last paragraph on traditional Mediterranean production systems, the results presented in this paper concern “conventional” breed of pigs or crossbreeds, i.e. animals issued from lines that have been genetically improved for growth rate and carcass leanness.

FEEDING

The feeding level (restriction), pattern (restriction-realimentation) and the protein:energy ratio of the diet, together with the genetic growth potential of pigs, determine the growth rate and the composition of weight gain at both whole body and muscle levels. These factors are therefore used to modify growth rate and/or carcass and muscle composition at slaughter.

Feed restriction

Restricted feeding (up to 35% compared to *ad libitum* feed intake) can be applied to reduce growth rate and thereby increase age at slaughter at a given body weight (BW). A 25% restriction in feed allowance during the growing-finishing period decreases growth rate by about 27% (Lebret *et al.,* 2001). Since body fat deposition rate highly increases with age, in contrast to protein deposition rate which remains almost constant during the growing-finishing period (Reeds *et al.,* 1993), feed restriction affects more fat than lean tissue deposition when applied during the finishing period. Therefore, restricted feeding leads to leaner carcasses compared with *ad libitum* feeding (Ellis *et al.,* 1996; Wood *et al.,* 1996; Lebret *et al.,* 2001). IMF deposition is also reduced by up to 25% in the m. *Longissimus* of restricted compared with *ad libitum* fed pigs (Candek-Potokar *et al.,* 1998; Lebret *et al.,* 2001). Consequently, eating quality can be adversely affected with lower tenderness and juiciness (Ellis *et al.,* 1996) even though some studies do not report any significant effect of feeding level on loin sensory traits (Wood *et al.,* 1996; Candek-Potokar *et al.,* 1998). Muscle fibre type composition and glycolytic potential as well as technological meat quality traits (pH1, pHu, drip loss, colour) remain generally unaffected by feed restriction (Candek-Potokar *et al.,* 1998, 1999; Lebret *et al.,* 2001).

Compensatory growth response

Compensatory growth response is a physiological phenomenon of accelerated final growth rate induced by a restricted food supply during the growing period, followed by *ad libitum* feeding thereafter. The level of animal response to this feeding strategy depends on the onset, duration and intensity of the feed restriction, and the onset and duration of realimentation (Campbell *et al.,* 1983). When restriction occurs during early growth (28-90 days), a full compensatory response can be observed at slaughter at 140 d (Therkildsen *et al.,* 2004). Besides, pigs exhibiting compensatory growth might have increased muscle protein turn-over and improved meat tenderness, compared with controls slaughtered at similar age and BW (Kristensen *et al.,* 2004; Therkildsen *et al.,* 2004).
At the whole body level, compensation in the rate - and often efficiency - of weight gain mainly results from an increase in adipose tissue and internal organ growth, but not from a higher carcass lean deposition, generally giving rise to similar carcass composition at slaughter in re-fed as in control pigs (Bikker *et al.,* 1996; Heyer and Lebret, 2007). In pigs, storage capacity for IMF (i.e. number of adipocytes) increases with age, whereas IMF deposition increases with energy intake (Gondret and Lebret, 2002). Therefore, we could hypothesize that increasing slaughter age and final energy intake through a restriction – re-alimentation feeding strategy could enhance final muscle lipid accretion and IMF content at slaughter. However, in a recent study, we could not demonstrate any positive effect of refeeding from 70 up to 110 kg BW after restriction from 30 to 70 kg BW, on muscle lipid deposition rate nor IMF content at final slaughter. Therefore, meat eating quality was not improved in compensatory pigs compared with controls (Heyer and Lebret, 2007). It was concluded that elevated IMF content and improved pork quality might be achieved by modifying the onset or duration of the restriction and realimentation periods.

It is worthy noting that in the traditional Mediterranean production system, local pig breeds are finished during autumn in forests of oaks or chestnuts. Due to their high consumption of acorns or chestnuts, which are rich in starch, pigs exhibit a compensatory growth characterized by a very high lipid accretion at both whole body and intramuscular levels (Lopez-Bote, 1998; Secondi *et al.,* 2007). In that case, the rearing conditions (advanced slaughter age, compensatory growth with acorns feeding during finishing) allow pigs to express their high genetic potential for IMF deposition, with subsequent positive consequences on the eating quality of pork and pork products.

Dietary protein level and protein:energy ratio

Diet composition, particularly the protein to energy ratio can be used to modify the composition of growth and increase IMF deposition. Indeed, feeding pigs *ad libitum* with protein or lysine-deficient but adequate energy diets during the growing or finishing phases has been shown to increase IMF content and improve meat tenderness and juiciness. Growth rate is reduced as a consequence of limited protein or lysine intake. However, back fat thickness or percentage of dissectible fat is also increased, even though the effect is much lower on carcass than muscle lipid deposition (Essén-Gustavsson *et al.,* 1994; Wood *et al.,* 2004). For example, Castell *et al.* (1994) reported values of 15.3 *versus* 14.9 mm back fat thickness (P<0.10) and 3.4 *versus* 1.4 % IMF (P<0.001) for pigs fed *ad libitum* a diet containing 13.3 or 17.6% crude protein, respectively.

By contrast, a progressive decrease in lysine to energy ratio combined with limited energy allowance (80% of the *ad libitum* level) all over the growing-finishing phase leads to extended growing-finishing period and older pigs at slaughter, in order to fulfil the requirements of the french Label Rouge quality label (minimum 182 d at slaughter). This feeding strategy highly increases IMF deposition but does not modify back fat thickness and carcass lean meat content, compared with controls fed *ad libitum* (Fig. 1) (Lebret *et al.,* 2001). On the contrary, a global feed restriction (75% of *ad libitum* level) over the same period leads to similar overall growth rate, but leaner carcasses and lower IMF content. Therefore, a progressive reduction in lysine:energy ratio together with limited energy intake seems to be a more efficient strategy to modify rate and composition of growth at both carcass and muscle levels for improved pork quality, rather than *ad libitum* distribution of a protein-deficient diet or, worst, feed restriction alone. Unfortunately, despite its large influence on muscle composition, we could not demonstrate any positive effect of our feeding strategy on pork eating quality, suggesting that IMF would not markedly influence eating traits within the range of concentrations observed in this study. Other muscle traits, such as myofiber type composition, as well as technological meat quality are generally unaffected by the dietary protein or lysine:energy ratio (Castell *et al.,* 1994; Essén-Gustavsson *et al.,* 1994; Lebret *et al.,* 2001).

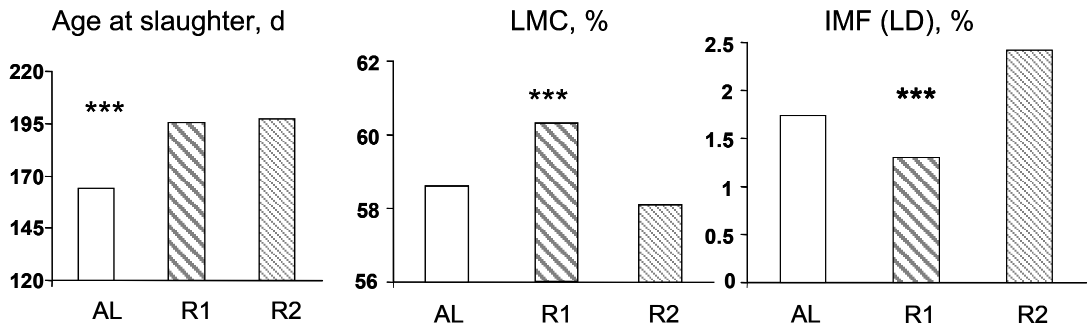

Figure 1. Influence of feed restriction (R1) or progressive decrease in dietary lysine:energy ratio (R2) compared to ad libitum feeding (AL) on age at slaughter, carcass lean meat content (LMC) and intramuscular fat (IMF) level in the m. *Longissiums* of Duroc crossbreeds (***: P<0.001) (Modified from Lebret *et al.,* 2001)

Dietary fatty acids and antioxidants

Fatty acid composition of pork can be easily manipulated through the feeding regime, as a consequence of the well known influence of the dietary fatty acids on fatty acid deposition in both subcutaneous and intramuscular lipids in pigs (Mourot *et al.,* 1991; Wood *et al.,* 2003). There has been an increased interest in recent years in manipulating lipid composition of pork to produce healthier meat, i.e. with increased n-3 PUFA (polyunsaturated fatty acids) level and decreased n-6:n-3 PUFA ratio (Legrand and Mourot, 2002; Wood *et al.,* 2003). Feeding sources rich in n-3 PUFA such as rapeseed oil and especially crushed linseed lead to increased n-3 PUFA level in meat, particularly for C18:3 and C20:5 (EPA), and C22:6 (DHA) to a lesser extent. The n-6:n-3 ratio is then reduced up to the target level of 4, compared to 10 in controls (Wilfart *et al.,* 2004). Even though these long chain n-3 PUFA are heat-sensitive, these authors demonstrated that the n-3 PUFA level and the n-6:n-3 ratio were not modified in cooked meat (loin roast) compared with raw muscle.

Increasing the n-3 PUFA concentration in pork must be accompanied with increased antioxidant concentration to prevent lipid oxidation and avoid unfavourable flavours in meat. Diet supplementation with vitamin E has been shown to prevent PUFA oxidation (Monahan *et al.,* 1990; Mourot *et al.,* 1991) and improve colour stability and water holding capacity during storage, through reduced cellular membrane damages (Monahan *et al.,* 1992; Cheah *et al.,* 1995). It is worthy noting that lipid composition and antioxidant levels in adipose and muscular tissues of pigs can be highly modified consequently to grazing during the rearing period (cf below).

REARING SYSTEM

Influences of pig rearing system on animal performance, carcass and meat traits result from the interactive effects of (i) housing conditions : floor type, space allowance, ambient temperature, outdoor access or free range rearing … that influence physical activity and feed requirements, (ii) feeding level and composition, and (iii) genotype, especially in specific production systems with local pig breeds.

Indoor housing conditions: ambient temperature, floor type and space allowance

The ambient temperature influences the energy requirements and the growth performance of pigs, the energy maintenance requirement increasing as temperature decreases. In pigs fed *ad libitum* to compensate for their higher energy requirement, Lefaucheur *et al.* (1991) reported that rearing at a low ambient temperature (12 vs 28°C) had no effect on carcass fat percentage, but strongly influenced its distribution with increased external fat at the expense of internal adipose tissues. Pigs raised at 12°C were also shorter and more 'squat', altogether indicating animal adaptation to the environmental conditions. The low temperature influenced fatty acid composition through higher monounsaturated and lower saturated and polyunsaturated FA proportions. Cold exposure enhanced the glycolytic capacity of the *Longissimus* muscle and led to lower initial and ultimate post-mortem pH, thus impairing technological meat quality, whereas IMF content was not affected (Lefaucheur *et al.,* 1991). By contrast, a smaller difference in ambient temperature (17°C vs 24°C) increased growth rate due to higher voluntary feed intake, but did not affect backfat and muscle thickness, or technological meat quality. Rearing at 17°C led to lighter and less homogeneous colour of dry-cured hams, but did not affect texture or flavour (Lebret *et al.,* 2002). On the opposite, warm exposure (31 vs 18.5°C) reduced growth rate as a consequence of the reduced feed intake of pigs, and decreased the monounsaturated FA level in back fat (Rinaldo and Le Dividich, 1991).

The floor type in pig buildings indeed modifies various other housing conditions, such as ambient temperature, space allowance, and the level of physical activity for animals, that all affect growth performance and carcass and meat traits. Compared to slatted floor (0.76 m²/pig), pigs reared on straw bedding (3.5 m²/pig) exhibited higher feed consumption, growth rate and carcass fatness (Beattie *et al.,* 2000; Gentry *et al.,* 2002a), the higher voluntary feed intake being likely explained by the lower ambient temperature and the easier access to the feeder provided in the "enriched" (straw-bedding) system (Lebret *et al.,* 2006a). The positive influence of enriched housing conditions on increased investigative activity of pigs is now clearly established (Petersen *et al.,* 1995; Beattie *et al.,* 2000) and may be interpreted as improved animal welfare. The possible effects of animal behavior during the rearing period on their physiological responses to stress at transport and slaughter, and consequently on pork quality, are of great interest (Terlouw, 2005). Several studies (Geverink *et al.,* 1999; de Jong *et al.,* 2000; Klont *et al.,* 2001) aimed to evaluate the effect of the enrichment of indoor environment (extra space and straw versus conventional) on pig behavior and physiology during preslaughter handling and subsequent meat quality. Although housing conditions have been shown to affect animal activity during transport and salivary cortisol levels both at the home pen and during transport, the differences were generally no longer significant at the end of the lairage period. This led to only small (Klont *et al.,* 2001) or non significant (Geverink *et al.,* 1999) effects on biochemical or technological meat quality traits.

Maw *et al.* (2001) assessed meat produced from different farms in Scotland, taken into account the effects of genotype and husbandry conditions (floor type, space allowance, air quality…). They demonstrated that bacon from pigs reared on straw bedding had higher greasiness and eating quality (flavor) scores to that from pigs reared on slatted or concrete floor without bedding. A possible explanation for the improved pork flavor could be a higher IMF content that would parallel the usually higher carcass fatness of pigs reared on straw bedding (Beattie *et al.,* 2000; Gentry *et al.,* 2002a), because of the positive genetic correlation between these two traits (Sellier, 1998).

Alternative housing system with outdoor access

Alternative pig rearing system with indoor space and free outdoor access constitutes an interesting intermediate situation between indoor-enriched and free range systems, as it positively influences the perception of pork by the consumers (Rainelli, 2001; Dransfield *et al.,* 2005) but with reduced drawbacks on labour conditions for the producer and production (feeding) costs, compared with free-range rearing. Moreover, it corresponds to regulations for improved quality pork, i.e. the 'Scharrel' pigs in the Netherlands and the Label Rouge Fermier in France, and to the European standards for housing of organic pigs.

An experimental evaluation of two production systems for growing-finishing pigs (synthetic line x (Large White x Landrace) crossbreeds, all free of the halothane-sensitive (n) and RN^- alleles) was conducted to evaluate animal welfare and health, growth performance, carcass and meat quality, and environmental impact (Lebret *et al.,* 2004, 2006a). Sawdust-shave bedding (1.3 m²/pig) with free access to a sheltered outdoor area on concrete floor (1.1 m²/pig) (O) was compared with a conventional system (totally slatted floor, 0.65 m²/pig, controlled ambient temperature at 22°C) considered as control (C). Concerning animal behaviour, pigs reared in the O system spent less time resting (58 *versus* 73%, P<0.001) and more time in investigative behaviour (30 *versus* 19%, P<0.001) particularly towards the bedding, compared with the C pigs, in agreement with Lyons *et al.* (1995) and *Beattie et al.* (2000). Sensitivity to respiratory tract pathologies as well as plasma haptoglobine level, an indicator of the inflammatory response, were lower for the O than the C pigs. Altogether, this suggests that the O system would improve animal welfare.

A comparison of air quality between the bedding (O system) and conventional rooms showed similar levels of dusts and ammonia, but a high decrease in the level of offensive odours determined by olfactometry in the indoor area of the O compared with the C system, that might lead to a better acceptability of this production system by citizens (Lebret *et al.,* 2004). Pigs reared in the O system exhibited higher growth rate due to their higher feed intake, higher back fat depth and lower lean meat content (Table 1). The O system did not influence the behavioural activities of pigs during lairage at the slaughterhouse, or plasma ACTH, cortisol and creatine kinase immediately after slaughter. This indicates that the rearing system (i.e., the prior experience of pigs) did not influence their behavioural or physiological response to pre- and slaughtering procedures, and the pattern of muscle peri- and post-mortem metabolism (Table 1) (Lebret *et al.,* 2004, 2006a). However, in a more stressful environment, the animal response to preslaughter handling could have differed according to their rearing conditions (Terlouw, 2005).

In the *Longissimus*, ultimate pH was not affected, but the O pigs had higher drip loss and higher IMF content. By contrast, a higher glycolytic potential and a lower ultimate pH were observed in the *Semimembranosus* and *Biceps femoris* muscles of O compared with C pigs (Table 1). Therefore, the influence of pig rearing system on muscle glycogen store or use and consequently ultimate pH is muscle-dependent, the ham muscles being more affected than the loin, confirming the results of Gentry *et al.* (2002a) and Bee *et al.* (2004). The higher glycogen level in the ham muscles of the O pigs might have resulted from their higher spontaneous physical activity, which was shown to enhance muscle oxidative capacity and thereby spare intramuscular glycogen (Petersen *et al.,* 1998).

Concerning eating quality, the O system increased meat juiciness (average score of 3.7 *versus* 3.4, for O and C meat, respectively, P<0.05), which may have resulted from its higher lipid content, whereas odour, flavour and tenderness remained unaffected (Lebret *et al.,* 2006a). Pig production system did not influence the overall appreciation of meat by consumers when no information on the pig production system was provided. But, awareness of the production system strongly influenced the perception of pork with 59% of the French consumers under study choosing the meat labeled 'outdoor' and 8% the meat labeled 'indoor' (34% inconsistent choices) (Dransfield *et al.,* 2005). These results highlight the differences between the "perceived" and "actual" quality of pork products issued from outdoor systems and conventional genotypes, as previously discussed by Edwards (2005).

Table 1. Effects of rearing conditions on performance, indicators of physiological response of pigs to preslaughter handling, carcass composition and muscle traits. (Reproduced with permission from B. Lebret, "Comparaison expérimentale de deux conduites d'élevage de porcs en croissance", Journées de la Recherche Porcine, 36, 53-62, published by Institut Technique du Porc, Paris, France, 2004)

	Rearing system		
	Conventional	Outdoors	Sign.[a]
Average daily gain, g/d	960	1045	***
Feed consumption, kg/d	2.71	2.94	**
Feed conversion ratio, kg/kg	2.83	2.82	ns
Slaughter weight (155 d), kg	109.6	116.6	***
Average backfat thickness, mm	18.5	20.9	**
Lean meat content, %	61.2	59.2	***
Plasma cortisol, ng/ml	42.2	49.9	ns
m. *Longissimus*			
pH 30 min	6.42	6.37	ns
pH 24 h	5.49	5.50	ns
Drip loss, 4 d post mortem	4.6	5.7	**
IMF, %	1.44	1.68	**
m. *Semimembranosus*			
pH 24 h	5.57	5.50	***

[a] ***: P<0.001; **: P<0.01; ns: P>0.05. [b] Winter replicate

Free range rearing

Several studies have been conducted to evaluate the effects free range rearing of "modern" pig genotypes on performance and meat quality. In these systems, pigs are submitted to various and changing climatic conditions; they are offered great space and environmental diversity, allowing physical activity and expression of investigative behaviour, and potential to forage for a range of different foodstuffs complementarily to the 'conventional' food provided. All these factors interact to determine the animal response in terms of growth and meat quality. Pigs raised outdoors are generally kept in large groups, thus avoiding or limiting the mixing of pigs from different pens during transport or lairage at the slaughterhouse, and its consequences on animal behaviour and meat quality. However, the occurrence of mixing during preslaughter handling depends on the group size and the management practices, and can vary between the systems – and the experiments – considered. It is therefore an important point to be considered when evaluating the influence of rearing system on pork quality, especially since the stress reactions of pigs to slaughter procedure can depend on their prior experience (Terlouw, 2005).

Many studies show that outdoor rearing in mild climate has only slight or even no significant effects on growth rate and carcass composition (Gentry *et al.,* 2002a,b), but reduced growth rate and back fat thickness have been observed for pigs reared outdoors in cold climates, particularly when average ambient temperature is below the thermoneutral zone (Enfält *et al.,* 1997; Sather *et al.,* 1997; Bee *et al.,* 2004). As for carcass traits, influence of extensive outdoor rearing on muscle composition, particularly lipid content varies with the actual rearing conditions of pigs (climate, feeding level). Both decreased (Enfält *et al.,* 1997; Sather *et al.,* 1997; Bee *et al.,* 2004) or similar (Nilzen *et al.,* 2001; Gentry *et al.,* 2002b) muscle lipid contents have thus been reported for outdoor compared with conventional reared pigs. However, it must be mentioned that grazing or the consumption of different feedstuffs by pigs reared outdoors strongly influences the fatty acid composition of animal tissues. For instance, the level of linolenic (18:3) and other n-3 PUFA is highly increased, and the n-6:n-3 ratio is decreased in meat from pigs reared on pasture compared with controls, as a consequence of the very high amount of C18:3 in the grass (Nilzen *et al.,* 2001; Bee *et al.,* 2004; Lebret and Guillard, 2005). The higher n-3 PUFA is accompanied by increased vitamin E deposition in both external fat and intramuscular lipids (Nilzen *et al.,* 2001), thus preventing further excessive lipid oxidation during meat storage (Andres *et al.,* 2001). Therefore, outdoor rearing beneficially modifies the nutritional quality of meat. In a similar manner, the consumption of grass or acorns during finishing of pigs in the traditional Mediterranean pig production systems highly influences the fatty acid profile of tissues and the subsequent quality of pork products (cf below) (Lopez-Bote, 1998).

Concerning the technological qualities of meat, Gentry *et al*. (2002b) and Bee *et al*. (2004) reported no differences in the rate and extent of *post-mortem* pH drop in the *Longissimus* of outdoor compared with indoor pigs, whereas Enfält *et al*. (1997) observed reduced ultimate pH and water holding capacity in the loin of outdoor reared pigs. Again, the consequences of outdoor rearing on muscle technological traits are likely muscle-dependent, with greater negative effects in ham than loin muscles (Gandemer *et al.,* 1990; Bee *et al.,* 2004). An important and often debated question is weather pigs reared in different environments cope differently with pre-slaughter stress, thereby leading to differences in meat quality. Terlouw et al. (2004) evaluated the behavioural and physiological responses of pigs to preslaughter mixing, depending on their rearing conditions (oudoor *versus* conventional). They showed that, when mixed, outdoor pigs exhibited lower fighting levels than indoor pigs, resulting in lower skin damage, higher pre and post-slaughter muscle glycogen level, and lower pH for the formers. Barton-Gade (2004) also reported less aggressive events and serum creatine kinase activity for outdoor than indoor reared pigs after mixing at loading, suggesting that mixing is more stressful for conventional than outdoor reared animals. Consequences on meat quality indicators remained low in the study of Barton-Gade (2004), but can be of greater extent when preslaughter handling conditions (high level of mixing of pigs from different farm pens) promote aggressive behaviour and physical activity (fights) during lairage in conventional reared pigs compared with a group of outdoor-non mixed pigs, a situation that can often be encountered in practical conditions (Lebret *et al.,* 2006b). Eating quality of loin meat issued from conventional genotypes reared outdoors in mild climate conditions and controlled (low stress) pre-slaughter handling is only slightly (improved tenderness, Gentry *et al.* (2002b) or even not modified (Gandemer *et al.,* 1990). In contrast, Enfält *et al.* (1997) reported decreased tenderness and juiciness of loin from outdoor reared animals, which could be explained by their lower lipid content and ultimate pH value.

Traditional Mediterranean production systems: Genotype X rearing system interactions

The traditional Mediterranean sylvopastoral system is based on local breeds that are extensively pastured in natural forests for the production of high value dry-cured products, in particular hams. These breeds exhibit slow growth rate, great fatness and mediocre conformation, and a greater predisposition to deposit oleic acid than conventional breeds (Edwards and Casabianca, 1997). In addition, in the 'traditional' systems, the finishing takes place during autumn in forests of oaks or chestnuts ('la dehesa'). The animals convert large quantities of acorns or chestnuts, which are rich in starch, into fat deposits at both whole body and intramuscular levels, resulting in very high eating quality (juiciness, flavour) of dry-cured products: 'montanera' finishing for Iberian pigs or 'montanheira' for the Portuguese Alentejano breed, and similar traditional finishing systems for Corsican, Basque or Gascon pigs in France; Cinta Senese and Nero Siciliano in Italy. Besides advantages of this extensive late fattening phase when considering the pork chain, in particular the quality of products, this traditional production system is also of major significance for the management of the forest heritage and the conservation of landscape: the 'dehesa' is a man-made ecosystem which becomes rapidly unproductive when abandoned. Therefore, in these traditional Mediterranean production systems, the pig production is deeply bound to the ecosystem and significantly collaborates in its preservation (Lopez-Bote, 1998; Edwards, 2005).

The use of local breeds together with the utilisation of the natural environment for the production of specific and high quality pork products is explicitly recognized through the "Protected Designation of Origin" (PDO) European label, even though all PDO terms and conditions for pork products do not necessarily involve local breeds. For example in Spain, 4 PDO labels ("Dehesa de Extremadura", "Guijuelo", "Huelva", "Pedroches") are based on pure Iberian pigs or their crossbreeds (see below). Other European pork PDO labels are based on pure local breeds: the Alantejano in Portugal ("Presunto de Barrancos") and projects that are underway in Italy with the Nero Siciliano and Cinta Senese, and in France with the Corsica, Basque and Gascon breeds. By contrast, white pigs are used for the production of PDO "Jamon de Teruel" in Spain, and many Italian PDO labels ("Prosciutto di San Daniele", "Prosciutto di Parma") are based on the tradition of heavy pigs from conventional genotypes raised in indoor systems.

The pig production systems for the production of dry-cured Iberian hams, which are indeed not unique but where different genotypes and rearing conditions can be encountered, are interesting examples of the positive consequences of genetic x environment interactions on pork quality. All these combinations are included in the PDO regulation ("Norma de Calidad"). Concerning genotype, pigs are issued from pure Iberian sows (including the various existing red and black lines) and pure Iberian, Duroc, Duroc-Jersey or their crossbreeds, as sire. Therefore, the “Iberian ham” denomination includes hams from purebred Iberian up to 50% Iberian-50% Duroc pigs. These crosses have been carried out (mainly with Duroc-Jersey) to increase prolificity and improve growth rate, feed efficiency and lean content, without serious damage to the quality characteristics of the meat products according to Lopez-Bote (1998), even though a recent study showed that differences in ham eating properties are noticeable by consumers (Ventanas *et al.,* 2007). Besides genotype, different feeding systems during finishing can be encountered (Lopez-Bote, 1998; Daza and Lopez-Bote, 2007):

- “Cerdo de bellota”: the late fattening phase (90-120 up to 140-160 kg) takes place on oak woodland pasture (‘montanera’) in specified regions for 2 to 3 months between November to January, which corresponds to the maturation period of acorns. During this phase, pigs must gain a minimum of 46 kg BW, and they are fed only the natural resources present on the land, i.e. mostly acorns (7 to 10 kg/d) and variable quantity of grass. The average growth rate of pigs is generally comprised between 750 and 1000 g/d during the montanera finishing period, which can therefore be considered as compensatory growth. The very high contents of starch (50% of dry matter (DM)) and fat (6-9% of DM) of the acorns lead to a very high accumulation of body lipids during this period, whereas the grass supply is an important source of protein (14-17% of DM) and compensates for the low protein concentration of acorns (4-6% of the DM). Moreover, the high linoleic (C18:1 n-9) acid (more than 60% of the FA) and the limited concentrations of linolenic and saturated FA of acorns, together with the high concentration of n-3 FA of the grass, modifies the lipid profile of Iberian pigs towards increased proportions of C18:1n-9 and C18:3, C22:5 and C22:6 n-3, and decreased levels of C16:0, C18:0 and C18:2 n-6 (Rey *et al.,* 2006). As mentioned above, grass is also an important source of α-tocopherol which prevents subsequent lipid oxidation during storage, together with other micronutrients present in the feeds ingested by the Iberian pigs (Rey *et al.,* 1997; Andres *et al.,* 2001).

- “Cerdo de recebo” (mixed system): pigs start the finishing period in the ‘montanera’ system where they gain at least 29 kg, and afterwards receive supplementary feed mainly based on cereals and leguminous plants. This production system is encountered in case of insufficient production capacity or too high stocking charge of the woodland.

- “Cerdo de pienso”: pigs are finished indoors or outdoors in free range systems and receive formulated feeds based on cereals and leguminous plants. This system gives the ability to produce Iberian pigs throughout the year, and has therefore led to the expansion of the Iberian pork industry.

The classification between ‘montanera’ or ‘recebo’ depends on the fatty acid composition of the subcutaneous adipose tissue, the thresholds being determined every year by the Ministry of Agriculture and the Interprofessional Association for Iberian pig production. For example, in 2003-2004, pigs were considered as ‘bellota’ for maximum levels of 21% C16:0, 9.8% C18:0, 9.5% C18:2, and a minimum level of 54% C18:1 (Daza and Lopez-Bote, 2007).

As for the pig production system, the process of Iberian dry-cured hams is also well adapted to the natural environmental conditions of the mountainous regions in the South west of Spain, with very long process duration in comparison to other meat products of the Mediterranean area (18-24 months of processing for hams). This of course participates in the specific characteristics of the Iberian pork products that have been recognized and are valued through the PDO label.

Within genotypes, studies have been carried out to describe variations in fat and muscle tissues characteristics and eating traits during the finishing regime: for example, Cava *et al.* (2000) and Rey *et al*. (2006) for Iberian pigs, Gueblez *et al*. (2002) for Basque and Gascon pigs, Pugliese *et al*. (2004a) for Nero Siciliano and Pugliese *et al*. (2004b) for Cinta senese pigs. As for the ‘bellota’ Iberian system, Secondi *et al*. (1992) showed that finishing of Corsican pigs on chestnut plantation leads to a compensatory growth response with very high fat deposition. They indeed demonstrated that the succession of a moderate followed by a fast growing period in extensive finishing conditions would be necessary to express the high potential for muscle lipid accretion of the Corsican pigs (Secondi *et al.,* 2007). During extensive finishing with chestnuts feeding, intramuscular fat content is increased from 1.9 up to 5.8% in the *Longissimus,* mainly due to accumulation of triglycerides (storage lipids). This is accompanied by an increase in the proportion of MUFA in muscle triglycerides from 42.6% up to 55% of the FA, and a decrease of PUFA from 16.6 up to 5.3% (Secondi *et al*., 1992). The type of finishing system strongly influences the quality of pork products, as shown by Cava *et al*. (2000) when comparing the sensory characteristics of dry-cured hams from Iberian pigs reared either in a free range based on acorns an pasture, or in confinement on a concentrate feed (Fig. 2).

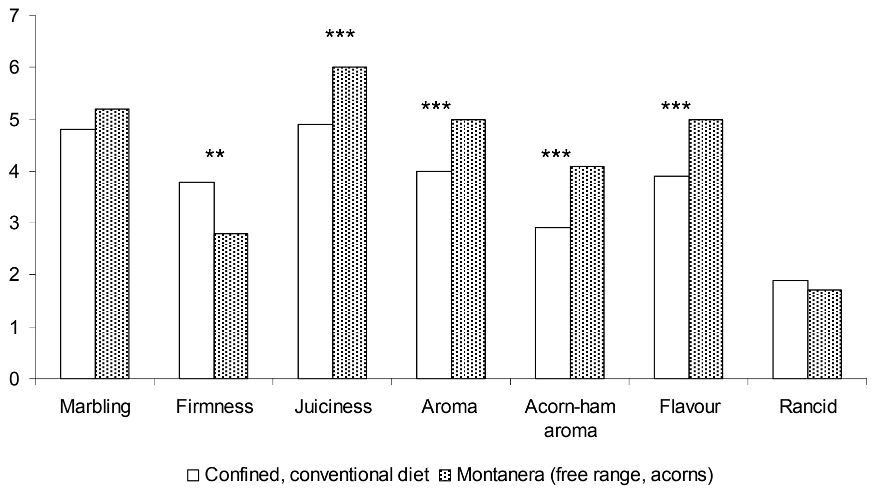

Figure 2. Influence of rearing system on the sensory traits of Iberian hams (**: P<0.01; ***: P<0.001) (modified from Cava *et al*., 2000)

Influence of the rearing system (and crossbreeding for Iberian ham production) is also noticeable by consumers: Ventanas *et al*. (2007) reported that dry-cured loin from pure Iberian pig finished outdoors on acorns and grass was preferred to those from animals reared indoors on concentrates, even though the mixed diet was enriched with MUFA and antioxidants, or to meat from Iberian X Duroc crossbreds, despite similar IMF contents. Altogether, these results demonstrate the occurrence of genotype x environment interactions on animal growth pattern, carcass and muscle properties, and their positive consequences for the sensory quality and the acceptability of pork products.

CONCLUSIONS

This article shows that both feeding and rearing system influence growth performance and carcass composition in pigs, through the relative growth deposition of fat and muscular tissues. Muscle composition can also be affected, in particular lipid content, thereby influencing pork eating quality. Other muscle components can of course impact pork eating quality, especially glycogen stores at slaughter and post-mortem muscle metabolism, that largely depend on pre-slaughter handling procedure, but also on the rearing conditions of animals (space allowance, ambient temperature, physical activity…). Therefore, meat quality can be manipulated through feeding and rearing systems, but studies generally show limited effects on sensory quality when using conventional (“improved”) pig genotypes. However, pigs from local breeds reared in extensive finishing conditions lead to high quality pork products, thereby demonstrating the positive genotype x environment interactions. These local breeds of pigs show a high potential for intramuscular fat deposition that can be expressed by their specific rearing conditions, but they exhibit very likely other differences in composition and ultrastructure of muscle that could impact pork quality, and are therefore interesting models for the studies on the relationships between muscle properties and subsequent pork quality. A better understanding of the relationships between muscle phenotypical traits at a “deep” level (muscle composition, metabolism, fibre typing, transcriptomics and proteomics approaches to characterize the expression of genes and proteins,…) and their relationships with eating quality traits should be achieved through the current European Q-Porkchain project, allowing further improvement of the quality or pork meat through rearing / genetic factors.

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PRELIMINARY RESULTS ON EXTENSIVE FREE RANGE REARING OF PIGS WITH ACCESS TO PASTURE INDICATE INFERIOR MEAT QUALITY AND IMPROVED FATTY ACIDS COMPOSITION

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SUMMARY - There's a growing interest for free-range rearing as it offers a niche market for consumers sensitive to animal welfare. Ten pigs (Large White/Landrace and Pietrain crosses, 5 gilts and 5 barrows) at age of 84 days, weighing 30.0 ± 0.9 kg were placed to outdoor rearing from May to November 2006. They were provided app. 4000 m^2 of pasture and a barn (2 m x 5 m). In addition to pasture grazing pigs received commercial feed mixture and pumpkins. Pigs were slaughtered at 280 days and 133.3 kg (growth rate of 550 g/day). The following day carcass and meat quality parameters were measured and samples of LD muscle and backfat taken for chemical and fatty acids analysis. High carcass lean meat percentage (61.5 %), low LD intramuscular fat (1.0 g/100g) and high iron content (633 µg/100g) were determined. LD muscle was noted rather pale (2.5 on a Japanese scale 1-6) in accordance with Minolta L* (57.3), a*(8.4), b*(5.0) values. High drip losses were observed after 24 and 48 hours (8.6 and 10.6 %, respectively). In comparison to literature data for conventional feeding higher contents of PUFA (26.1 g/100g total fatty acids), lower n-6/n-3 PUFA ratio (7.0) and higher P:S ratio (0.72) were observed. In our case free-range rearing with restricted feeding indicates inferior pork technological quality and improved fatty acid composition.

Key words: Pigs, free range rearing, meat quality, fatty acids.

INTRODUCTION

Free-range rearing represents an alternative way to the conventional and/or intensive fattening of slaughter animals. It offers a new niche market, since a modern generation of consumers chooses meat products not only according to price and eating quality, but considers also the ethical quality of meat, such as animal welfare issues and environmental impact of the production system (Garnier *et al*., 2003). It is also believed that this type of rearing improves the value of pork considering both sensory and nutritional (health) value. Therefore it became important to investigate the influence of alternative rearing systems on pig production and quality of pork (Nilzen *et al*., 2001). In view of the balanced diet the quality of meat lipids is of primary importance. The majority of lipid fatty acids of pig meat is monounsaturated (MUFA, mostly C18:1) and saturated fatty acids (SFA, mostly C16:0 and C18:0), but also polyunsaturated fatty acids (PUFA) (C18:2, C18:3) are notable (Žlender and Gašperlin, 2005). Increased dietary intake of PUFA, n-6 and, especially, n-3 fatty acids is beneficial to human health, as it reduces plasma cholesterol levels and thereby lowers the risk of cardiovascular disease (Van Oeckel *et al*., 1997). Nutritional quality of lipids indicates relation between PUFA and SFA (P/S index with recommended value above 0.4) and n-6/n-3 PUFA ratio, recommended values are below 4 (Wood *et al*., 2003). As monogastric animals, pigs have adipose tissues with fatty acid composition that closely resembles that of the dietary fatty acids intake. Many previous investigations indicate that free-range rearing with access to green feed alter the fatty acid composition by increasing the ratio of unsaturated fat, being therefore more beneficial in human nutrition (Dufey, 1995, Jakobsen, 1995, Johansson *et al*., 1996, Nilzen *et al*., 2001). Because of lower melting points, increased proportion of unsaturated fatty acids may also exert negative impacts such as excessive softness and oiliness of subcutaneous fat/carcasses, which can cause problems for the meat processor and retailer (Wood *et al*, 2003, Smith *et al*., 2004). In addition, an increased amount of PUFA in muscle makes the tissue more susceptible for lipid oxidation and may lead to the occurrence of colour changes and rancidity (Chan, 2004). Some literature data mention the negative impact of outdoor rearing on meat technological quality, especially water holding capacity, ultimate pH and colour (Enfält *et al*., 1997, Nilzen *et al*., 2001, Edwards, 2005), whereas in some other studies (Warris *et al*., 1983, van der Wal *et al*., 1993, Gentry *et al*., 2004), no such effects were observed. According to Oksbjerg *et al*. (2005) who compared different out/indoor rearing and *ad libidum*/restrictive feeding regimes, the pigs with restricted feeding regime showed inferior meat quality, but no difference in meat quality between out- and indoor rearing was noted. Traditional extensive breeds are considered more appropriate for outdoor rearing than high productive modern breeds, where higher stress level induced by poor adaptation to climatic and nutritional factors can reduce the quality of meat (Edwards, 2005).

MATERIAL AND METHODS

The material of our study consists of ten pigs (Large White/Landrace×Pietrain crosses, 5 gilts and 5 barrows). All animals were free of mutation on RYR1 gene. At age of 84 days, and weight of 30.0 ± 0.9 kg the pigs were placed to outdoor rearing from the middle of May to the end of November 2006. They were provided 4000 m^2 of pasture and a straw bedded barn ($2 \times 5m^2$). In addition to pasture grazing, the pigs received commercial feed mixture, which consisted of grower diet (0.7 kg/animal/day, 13.6 MJ/kg, 18.0 % crude protein) for the first 60 days, a finisher diet (1.8 kg/animal/day, 13.0 MJ/kg, 16.0 % crude protein) until slaughter and for the last 3 months 2 kg of pumpkins (*Cucurbita pepo*) per animal per day were offered. Pigs were fed a restricted diet in order to stimulate pasture grazing. The health and behaving of pigs was regularly inspected. Pigs were slaughtered according to the commercial slaughter procedure at the age of 280 days. A week prior to slaughter they attained average weight of 133.3 kg (growth rate app. 550 g/day). After the slaughter warm carcasses were weighed (dressing percentage was calculated as a ratio of warm carcass weight and live weight) and chilled at 4°C. The following day backfat and *Longissimus dorsi* (LD) muscle thickness was measured using HGP4 Hennessy grading probe (Hennessy Grading Systems Ltd., Auckland, New Zealand) with puncture between the second and third last rib 7 cm laterally from the carcass split line. The carcasses were cut transversally at the level of last rib and a digital image of the cross-section was taken using a camera (Canon PowerShot G3, Canon Inc., Tokyo, Japan). LD muscle area, corresponding fat area and belly leanness were determined on images using LUCIA.NET 1.16.5 software (Laboratory Imaging s.r.o, Prague, Czech Republic). The hind leg was cut off the carcass between 6^{th} and 7^{th} lumbar vertebra and shank removed. The weight of leg (ham) was recorded before and after the removal of the skin and subcutaneous fat and ham leanness (%) calculated as the ratio between muscle with bones and whole ham weight. Tarsal joints (*Articulus tarso-metatarsicus*) were also inspected for possible lesions. A 2.5 cm thick slice of *M. longissimus dorsi* and adjacent backfat at the level of last rib was taken for meat quality and meat and fatty acid composition analysis. The measurements of color and pH were taken on the freshly cut surface of LD. Color of LD was assessed using 6 point Japanese color scale (Nakai et al., 1975). Color parameter measurements (CIE L*, a* and b*) were taken in triplicate using a Minolta Chroma Meter CR-300 (Minolta Co. Ltd, Osaka, Japan) with an 11 mm diameter aperture, D_{65} illuminant, calibrated against a white tile. Muscle pH was determined in two replicates in the central area of the *LD* using a MP120 Mettler Toledo pH meter (Mettler-Toledo, GmbH, 8603 Schwarzenbach, Switzerland) fitted with a combined glass electrode (InLab427) and previously calibrated at pH 4.0 and 7.0. Drip loss measurement was performed according to the method (EZ drip loss) published by Christensen (2003). Drip loss was determined after 24 and 48 hours storage at 4°C and expressed as a percentage of the initial weight. For fatty acid composition determination fatty acids methyl esters (FAME-s) were prepared from the sample tissue using the method of Park and Goins (1994). For separation of FAME an Agilent 6890 series GC instrument (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with an Agilent 7683 Automatic Liquid Sampler, an Agilent 7683 split/splitless injector, a flame-ionization detector (FID) and a capillary column Omegawax 320, 30 m × 0.32 mm i.d. (Sigma Aldrich Biotechnology L.P., St. Louis, MO, USA) was used. Agilent GC ChemStation Plus software was used for data acquisition and processing. Separated FAME-s were identified by comparison with the retention times of pure standards and reported as percentages of total fatty acids.

RESULTS AND DISCUSSION

Present study was preliminary with the aim to feed pigs restricted amount of concentrate in order to stimulate intake from grazing. Considering their age (280 days), the animals exhibited low body weight (low growth rate) due to low energy feeding regime, which is reflected in their body composition (Table 1). Pigs had large LD muscles (average thickness was 62.4 mm and cross-sectional area 59.7 cm^2) and little backfat (13.6 mm at 2/3 last rib, backfat area at last rib 14.1 cm^2) resulting in high carcass meat percentage (61.5 %). Hams weighed 13.6 kg and were also very lean (87.3 %). Reduced levels of concentrate feeding in combination with roughage intake explains low daily gain in growing pigs, which according to Edwards (2005) results in increased lean meat content and reduced intramuscular fat, but also the breed/crossing may have had an influence. Affentranger *et al*. (1996) comparing growth and carcass characteristics of different pig crosses (Pietrain, Large White and Duroc) under different feeding regimes, concluded that Pietrain crosses were the most affected by the restricted feeding regime (mostly reduced daily gain, subcutaneous and intramuscular fat and feed conversion ratio).

Table 1: Carcass and meat quality properties of pigs (n=10) on restricted feeding regime with pasture.

Carcass traits	Mean ± s.d.	Meat quality traits	Mean ± s.d.
Live weight, kg	133.3 ± 12.0	pH_{24}	5.53 ± 0.03
Carcass weight, kg	105.5 ± 10.3	LD colour (1-6)	2.5 ± 0.4
Dressing, %	79.1 ± 1.4	Minolta L*	57.3 ± 3.9
LD thickness[a], mm	62.4 ± 4.0	Minolta a*	8.4 ± 1.1
Backfat[a], mm	13.6 ± 3.7	Minolta b*	5.0 ± 1.7
Lean meat, %	61.5 ± 4.4	Drip loss 24h, %	8.6 ± 2.2
LD area[b], cm^2	59.7 ± 5.8	Drip loss 48h, %	10.0 ± 2.1
LD fat area[b], cm^2	14.1 ± 5.6		
Ham, kg	13.6 ± 1.1		
Ham(meat+bones), kg	11.8 ± 0.8		
Ham leanness,%	87.3 ± 3.2		

[a]measured with HGP probe on the level of 2/3 last rib
[b]at the level of last rib

Technological characteristics of LD muscle of pigs in the present study demonstrate inferior quality. The low ultimate pH value (5.53), low subjective colour score (2.5) and high Minolta L* (57.3) all support this statement. The study of Oksbjerg *et al*. (2005) proved lower pigment content in the meat of outdoor reared restrictively fed pigs, but they observe no significant differences in L* value between the rearing systems. The drip loss in the present study was remarkably high (8.6 and 10.0 % for drip loss after 24 and 48 hours, respectively). Similarly, Nilzen *et al*. (2001) indicated inferior water holding capacity in connection with outdoor rearing (indoor vs. outdoor rearing resulted in 5.8 % vs. 7.0 % drip loss, respectively). Gandemer *et al*. (1990) who also reported a lower water holding capacity in outdoor reared pigs suggested that lower water holding capacity could be the consequence of greater pre-slaughter stress. In confirmation of these facts, the review of Edwards (2005) mentions that a significant number of investigations report higher drip loss, suggesting greater susceptibility of outdoor reared pigs to pre-slaughter stress. The reason for inferior meat quality may in our case also lie in the unsuitable choice of crossing, since the animals for outdoor rearing must be robust enough to withstand the rigours of climate and less nutritive diet. If nutritional and rearing requirements for the specific breed are not met, both welfare and growth rate will suffer, and these, in turn, can impact adversely on meat quality. Traditional breeds or more robust breeds or crosses (e.g. Duroc) are recommended while the Pietrain breed is less appropriate (Edwards, 2005).

The results of chemical analyses of LD muscle (Table 2) showed low intramuscular fat content (1.0 %) in accordance with the high carcass leanness (61.5 %). Compared to the data from Slovenian food composition tables (Golob *et al*, 2006) for pig LD, pigs in the present study had LD muscle with higher crude protein content (24.2 vs. 21.2 %). The results of other studies (Dworschák *et al*., 1995, Enfält *et al*, 1997) are also reporting higher crude protein content in pigs reared outdoors. On the contrary, Nilzen *et al*. (2001) reported higher crude protein in indoor reared animals. Considering the content of the trace elements, only the content of iron showed considerably higher values (0.64 mg/100g of LD muscle) when compared to the iron content of conventionally reared pigs (Slovenian food composition tables, Golob et al., 2006). Possible explanation for this can be higher age at slaughter, intake of green feed and soil ingestion on the pasture.

Table 2: Chemical composition of LD muscle of pigs (n=10) on restricted feeding regime with pasture.

Constituent		Mean ± s.d.	Constituent		Mean ± s.d.
Dry matter	g/100g	26.3 ± 0.6	Ca	mg/100g	6.33 ± 0.52
Moisture	g/100g	73.7 ± 0.6	Mg	mg/100g	27.83 ± 0.98
Crude protein	g/100g	24.2 ± 0.6	K	mg/100g	366.17 ± 2.48
Ash	g/100g	1.15 ± 0.08	Na	mg/100g	59.00 ± 4.43
Intramuscular fat	g/100g	1.00 ± 0.37	P	mg/100g	221.67 ± 2.88
			Cu	mg/100g	0.10 ± 0.02
			Fe[a]	mg/100g	0.64 ± 0.78
			Zn	mg/100g	1.23 ± 0.07

[a]compared to the Slovenian food composition tables (Golob et al., 2006; 0.47 mg/100g), the iron content is much greater in the present study.

The fatty acid composition of subcutaneous adipose tissue and intramuscular fat are presented in Table 3. The adipose tissue of pigs from the present study contained, compared to the Slovenian food composition tables for conventionally reared pigs (Golob *et al.*, 2006), lower total SFA content, higher content of total PUFA, n-6 PUFA, n-6/n-3 PUFA ratio and increased total PUFA/SFA ratio. While higher PUFA and PUFA/SFA ratio can be considered as beneficial, higher n-6/n-3 PUFA ratio is not a positive fact, from the nutritional point of view. More unsaturated fatty acids means less appropriate raw material for meat products (Wood *et al.*, 2003, Chan, 2004, Smith *et al.*, 2004).

Table 3: Fatty acid composition of subcutaneous adipose tissue (backfat) and intramuscular fat of LD muscle in pigs (n=10) on restricted feeding regime with pasture.

			SI food composition tables[b]	
g/100g of total fatty acids	Backfat	Intramuscular fat	backfat	Intramuscular fat
total SFA	39.8 ± 1.4	36.1 ± 0.6	46.2	42.6
total MUFA	46.6 ± 2.1	37.8 ± 5.7	47.1	46.7
total PUFA	13.6 ± .2.5	26.1 ± 5.9	6.8	13.1
n-3 PUFA	1.8 ± 0.6	3.3 ± 1.0	1.7	0.9
n-6 PUFA	11.7 ± 2.0	22.7 ± 4.9	5.0	12.1
n-6/n-3 PUFA ratio	6.6 ± 1.0	7.0 ± 0.9	2.9	13.7
PUFA/SFA ratio	0.34 ± 0.07	0.72 ± 0.17	0.15	0.31

[b]Slovenian food composition tables. Meat and meat products (Golob *et al*, 2006).

In comparison to the data of Slovenian food composition tables, in the present study the fatty acid composition of intramuscular fat of LD muscle contained less SFA, more MUFA and PUFA (n-3 PUFA and n-6 PUFA); the n-6/n-3 PUFA ratio was almost half smaller. PUFA/SFA ratio was considerably higher (0.72 vs. 0.31 respectively) and closer to the values mentioned by Enser *et al*. (1996) for conventionally raised pigs (7.2 for n-6/n-3 PUFA ratio and 0.58 for PUFA/SFA). Due to the low intramuscular fat content it is likely that high PUFA content is related to high proportion of phospholipids, as they contain higher amounts of PUFA (Wood *et al*., 2004). In the same manner, the breeds/genetic types of animals with low muscle lipid content will have higher proportions of PUFA (Wood *et al*., 2007). Monin *et al*. (2003) proved higher intramuscular PUFA content in Pietrain breed compared to Large White, which could also explain partly high PUFA in our study. The addition of grass pasture (and pumpkins), which contain high proportion of unsaturated fatty acid, could also contribute to the increase of PUFA (Wood *et al*., 2003) as some fatty acids, like for instance 18:2n-6, are entirely derived from the diet (Wood *et al*., 2007). Some authors point out that restricted feeding alone induces higher unsaturated fatty acids content. According to Oksbjerg *et al*. (2005), who compared restricted and *ad libitum* feeding and outdoor/indoor rearing, only the feeding regime affects fatty acids composition.

The inspection of tarsal joints did not show any lesions. The general health of animals was good. Exercise and fresh air in pleasant climatic conditions are considered to reduce infection pressure in outdoor reared pigs (Edwards, 2005). From the welfare point of view, the herbage intake is considered beneficial since it promotes satiety in conditions of limited food intake.

CONCLUSIONS

In the present study outdoor rearing on pasture with restricted concentrate intake resulted in slow growth, lean carcasses and inferior pork technological quality (pale colour, high drip loss). The meat of pigs from this study had high content of polyunsaturated fatty acids beneficial from human nutrition point of view. Present study was preliminary serving more as a basis for further experiments. In the future, a control group of pigs for comparison and a genotype that is more adequate for extensive rearing should be used. Better planning of the daily rations will also be necessary, as poor adaptation to the restricted feeding regime along with the possible effect of the rearing environment or pre-slaughter conditions could be the origin of the stress leading to low meat quality.

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Trial to Study Possible Effect of Acorns Source (Green Oaks *vs* Cork Oaks) on Traditional Pork Products

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SUMMARY - One farm with green oaks and another with cork oaks were chosen. Aiming to minimize weather effects and to provide swine equivalent pastures, under oak canopy, density of trees was similar and geographic area limited within Ourique County. Alentejano hogs, born and raised inside the region and recorded in the breed herd book, were fattened exclusively under oaks. Chemical composition of acorns from green oaks (AG) and from cork oaks (AC) were analysed including on fatty acids profile. Pigs were weighted 3 times during fattening period and ecographies were performed to evaluate back fat thickness (BF) and loin depth (LD). After slaughter samples of back fat were taken and analysed on chemical crude composition and fatty acids. Weight losses of hams were recorded along ripening period. Chemical composition of AG and AC were different. Considering percent of DM, we got for AG *vs* AC: NDF 24.9 *vs* 29.8 ($p<0.001$); CP 3.67 *vs* 5.57 ($p<0.05$); Lipids 8.00 *vs* 6.20 ($p<0.05$). Fatty acids profile have shown higher percentage of saturated on AG ($p< 0.001$) and exhibit higher percentage of unsaturated on AC ($p<0.001$). Acorns type, AG *vs* AC, didn't affect significantly ADG neither the BF, but LD was deeper in hogs fattened with AC. Fatty acids of pigs fattened with AG *vs* pigs fattened with AC reflected the feed influence (C16:0, AG 19.53 *vs* AC 18.62 $p<0.001$; C18:0, AG 8.91 *vs* AC 8.43 $p<0.05$; C18:1, AG 56.09 *vs* AC 55.17 $p<0.001$; C18:2 AG 8.23 *vs* AC 10.42 $p<0.001$). Total hams losses during ripening period were not significant affected by feed intake.

Key words: Acorns, Alentejano pigs, fatty acids, sylvo-pastural.

INTRODUCTION

Green oak (*Quercus rotundifolia*) and cork oak (*Quercus suber*) together with other *Quercus* are the base of Mediterranean forests in Southwest Iberian Peninsula (about 5 million ha). This agro-sylvo-pastoral system, used time along, established a sustainable ecosystem (Tirapicos Nunes, 1993).

Alentejano swine breed is considered the best animal user of natural feedstuffs under oak canopy, between November and February. Little information has been published about the effect of acorns origin (green *vs* cork) on pig performance, meat and traditional products characteristics.
Quality of dry cured products has been related with feed intake, during last finishing period (Freitas, 1998 and Neves, 1998). This trail main goal was just to contribute for a better knowledge on: (i) acorn type influence on final characteristics of PDO Portuguese pork products, besides (ii) growth of pigs during "montanheira" period.

MATERIAL AND METHODS

System

Two pig farms within same region were chosen to perform fattening of Alentejano pigs. Livestock density was identical and it was in accordance with PDO rules (1 to 1.5 animals per ha). Each farm was different considering oak types of trees present, farm B exclusively with green acorns and the farm L exclusively with cork acorns

Animals

Seventy three hogs, sex neutralized, were engaged in this research, thirty four in farm B and thirty nine in farm L. Pigs ages were about 15 months at beginning of fattening period under oaks. Hogs were razed under rangeland conditions on an acorn plus grass diet during three months, without extra feed supply.

Procedures

Both acorn type fruits were sampled twice (1st - beginning December; 2nd – beginning January) in each farm.

Laboratory analyzes of acorns were performed on: protein; lipids (as % of DM); fatty acid composition. Pigs were weighted at beginning middle and end of fattening period. Inside the scale, 30% of animal were submitted to ultrasound measure of back fat thickness (BFT) and *Longissimus dorsi* depth (LDD). After slaughtered, back fat samples were picked (same place, last lumbar vertebra level) from pigs in order to process later analyzes to determine fatty acids profile (Campaniço e Nunes, 2006). Hams were weighted 4 times during ripening period to determine hams losses time along.

Statistics

Data were analyzed by ANOVA, according to the mathematic model:
$Y_{ij} = \mu + Farm_i + \varepsilon_{ij}$

RESULTS

Table 1 shows chemical composition of both acorn types, considering the amount of acorns eaten per swine each day (7 kg to 10 kg) these slight differences can be important in meat characteristics.

Regarding the influence of feed intake we can see that in average there are no differences in ADG between farms, if total period is considered, but as can be seen in table 2, pigs grown faster in L farms during first half period perhaps because L acorns ripened and fall early (Almeida *et al*, 1992).

Protein content, higher in L acorns, can explain the difference in *Longissimus dorsi* depth as shown in fig 1.

Table 1. Acorns chemical composition (least square means±sem)

	Green acorns	Cork acorns	SL
Protein (%DM)	3.67 ± 0,78	5.57 ± 0.8	*
Lipids (%DM)	8.00 ± 0.58	6.20 ± 0.58	*
Fatty acids (1)			
Palmitic (C16:0)	15.28 ± 0.43	14.68 ± 0.43	NS
Estearic (C18:0)	3.27 ± 0.43	1.77 ± 0.43	*
Oleic (C18:1)	63.25 ± 0.56	59.10 ± 0.56	***
Linoleic (C18:2)	15.63 ± 0.47	20.42 ± 0.47	***
Linolenic (C18:3)	0.82 ± 0.18	1.73 ± 0.18	**
Saturated	19.01 ± 0.22	17.06 ± 0.22	***
Unsaturated	81.14 ± 0.22	83.17 ± 0.22	***
Monounsaturated	64.11 ± 0.58	60.37 ± 0.58	***
Polyunsaturated	17.04 ± 0.69	22.80 ± 0.69	***

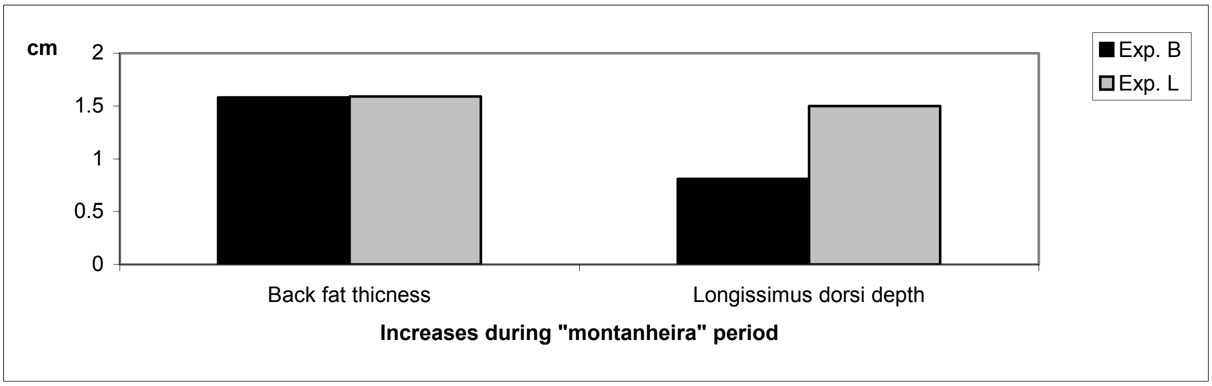
(1) As % of identified methyl esters; *- p<0,05; ** - p<0,01; *** - p<0,001; NS - No Significant

Table 2. Pigs growth performance

ADG	Exp. B	Exp. L	Sig
ADG I (g)	936.56 ± 36.76	1046.88 ± 43.63	***
ADG II (g)	930.64 ± 32.25	778.28 ± 38.28	***
ADG III (g)	933.35 ± 27.88	912.13 ± 33.09	NS

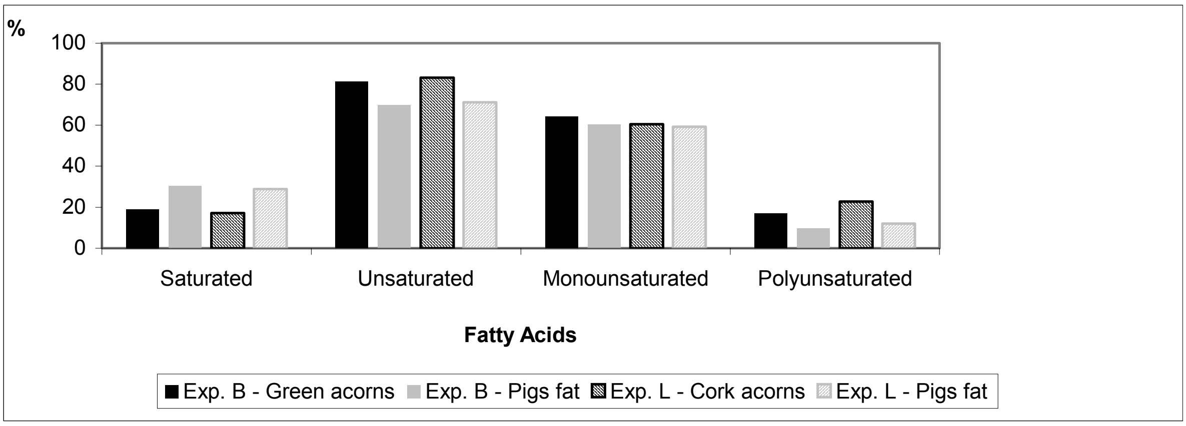
ADG I : average daily gain from beginning to half trial; ADG II: average daily gain from half to trial end; ADG III: average daily gain from beginning to trial end; *** - p< 0,001; NS - No Significant

Figure 1 Graphics based on ecographies



Fatty acids profile is considered to be very important to compare “montanheira” dry cured hams. Regarding the results obtained in this trial we can observe (fig. 2): (i) hogs raised in both farms presented adequate fatty acid profile considering PDO recommendations; (ii) differences in acorns seem to affect in same way fatty acids profile of raw material.

Figure.2 Fatty acids composition of acorns and fat.



CONCLUSIONS

- Comparing chemical composition of acorns, cork acorns were different from green acorns.

- Differences on acorns were reflected on meat and fat characteristics.

- Ham yield seem to be slightly affected by the type of fruits intake.
- Both acorns drive to obtain raw material and products within parameters established by certification official authorities for PDO Portuguese pork products.

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COMPARATIVE STUDY ON COLOSTRUM PRODUCTION AND COLOSTRUM COMPOSITION IN ALENTEJANO SWINE BREED AND LWxLR SOWS – PRELIMINARY RESULTS

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SUMMARY- This study aimed at comparing the colostrum composition and production of Alentejano Swine Breed (AL) to modern sows (LW x LR) (LL). Ten sows from each genotype were used. All farrowings were attended. Colostrum samples were collected at birth of the first piglet and at regular intervals during 36 h after the onset of farrowing. One sample of milk was also collected at d 15 of lactation. Piglets were weighed at birth and at 24 h of age. AL sows had lower gestation length ($p < 0.001$) and litter size ($p < 0.05$). Piglets from AL sow were lighter at birth ($p < 0.001$), gained less weight ($p < 0.001$) and consumed less colostrum ($p < 0.001$) than LL piglets between birth and 24 h of age. AL sows produced less colostrum ($p < 0.001$) than LL sows. Within each genotype colostrum consumption of piglets was dependent on birth weight ($p < 0.001$). Production of colostrum was dependent on litter weight (piglets born alive) at birth but not on litter size. Mortality rate between birth and weaning averaged 24.3% for AL piglets and 14.3% for the LL piglets, with most of losses occurring within 3 days after birth. In both genotypes, piglets dying before weaning were lighter at birth and consumed less colostrum than survivors. Further studies are required to determine the respective role of birth weight and colostrum consumption in post-natal mortality and to determine the immune quality of colostrum.

Key words: alentejano swine breed, colostrum, piglets, mortality

INTRODUCTION

The Alentejano swine breed is known to be less prolific while piglets mortality between birth and weaning is higher when compared to improved genotypes raised under intensive systems (Charneca, 2001). Colostrum is essential for piglet survival and growth, providing the piglets with immunoglobulin and energy during the first hours following birth (Le Dividich *et al*. 2005). Colostrum production of the sow and consumption of the piglets estimated indirectly from the change in piglets body weight between birth and 24h are very variable (Le Dividich *et al*., 2004; Devillers *et al*., 2005). The colostrum composition changes very rapidly towards that of milk during the first 24-36 hours post-partum (Klobasa *et al*., 1987).

Colostrum intake in piglets depends on both the ability of the sows to produce colostrum and on that of the piglets to extract the colostrum from the mammary glands which is influenced by the piglet body weight and piglet vitality (Devillers *et al*., 2007). However there is little scientific data on the composition or on both the production of the sow and consumption of the piglet from Alentejano swine. In this study we report preliminary results on colostrum composition, production of the sows and consumption of the piglets when AL sows are compared to modern sow raised under the same farrowing and nursing environmental conditions.

MATERIAL AND METHODS

The experiment was carried out in the Experimental Centre of Mitra – University of Évora – Portugal.

Animals: Ten Alentejano breed sows (AL) and 10 Large-White x Landrace (LL) sows and their litters were used. The Alentejano sows were mated by AL boars whereas Large-White x Landrace

(LL) sows were mated by a Large-White boar. Average parity was 3.2 ± 0.8 and 3.6 ± 0.8 for Al and LL sows, respectively.

Housing. The LL sows were always kept in indoor swine facilities. During gestation they were raised in groups (3-4 sows per group) on solid concrete floor. AL sows were raised as were LL sows during the first month of gestation. After ultrasonic confirmation of gestation they were moved to an outdoor park (4.5 ha of total area), where they had access to floorless arks. Seven days before the expected farrowing date, all sows were moved to the farrowing house and placed in individual conventional farrowing crates with concrete slatted floor in the sows' area and plastic floor in the piglets areas. Piglets were provided with local heating consisting of one infra-red lamp (175W) suspended above the creep area. Piglets were weaned at 28 days. In all facilities and parks, sows had free access to water from low-pressure nipple-drinkers.

Feeding. During gestation, the LL sows were fed twice a day with ~3 Kg of commercial diet containing (per Kg diet) 3200 Kcal DE, 160g CP and 7g lysine. AL sows were fed twice a day ~2.0Kg of the same commercial diet during the first month of gestation. In the outdoor pasture park they received the same amount and type of feed, but given once a day. Care was taken to spread the feed in the field. In the farrowing day no feed was provided to the sows and thereafter the lactation diet containing (per kg diet) 3300 Kcal DE, 170g CP and 10.5g lysine was gradually increased until *ad libitum.* Individual feed intake was not determined. However, during gestation sows were grouped on the basis of body weight and age in order to reduce competition and hence to avoid large differences in feed intake. Piglets had access to a creep feed containing (per Kg diet) 3400 Kcal DE, 190g CP and 12.4g lysine, starting on d14 of lactation. Piglets were weaned at 28 d of age.

Farrowing surveillance and samples collection. All farrowing were supervised but none was induced. Soon after birth, piglets had their umbilical cord cut at about 10-12cm from the navel after which they were identified (ear tag) roughly dried, weighed and returned to the sow. These operations were performed very quickly, usually within 2 min of birth. At 24 hours after birth, piglets were re-weighed. Piglets dying during the nursing period were weighed and approximate death time recorded. Colostrum consumption of the piglets between birth and 24 h of age was estimated according to the prediction equation of Devillers *et al.* (2004).

Colostrum (50-100ml) was collected from at birth of the first piglet and at 3, 6, 12, 24 and 36 hours after the birth of the first piglet, immediately filtrated on gauze and stored at -20ºC until they were analyzed. Milk samples were also collected at d15 of lactation. Sows were intramuscularly administered oxytocin (20 IU) to induce colostrum and milk release from 3 h onwards. Colostrum or milk letdown typically occurred 3 to 4 min later.

Analytical procedures. Dry matter (DM) of colostra and milk was determined after drying at 102ºC until constant weight. Crude protein (CP) was determined according to Dumas method (CP= N x 6.38) using a LECO FP-528 Nitrogen/Protein Determinator. Total lipids (LIP) content was determined according to Gerber method (AOAC, 1990) and lactose was determined using a commercial kit Boehringer Mannheim ® Lactose/D-Galactose (ref. 0176303).

Statistical analysis. All data were analysed using the NCSS (2001) software. Sows reproductive and productive traits data were analysed using the general linear model (GLM) procedure with the one-way analysis of variance (ANOVA) using genotype as fixed effect. Litter weight gain and colostrum production of the sow, were also analysed by covariance using litter size and litter weight (live born piglets) as covariates. Colostrum and milk data were analyzed using a split-plot ANOVA with genotype as the main effect and sampling time as a repeated measure. Tukey-Kramer test was used to separate means. Newborn piglets' weights, weight gain from birth to 24h of age, colostrum intake and data were analysed using the general linear model (GLM) procedure with the one-way analysis of variance (ANOVA) using genotype as fixed effect. Colostrum intake (g/Kg BW) was also analysed by covariance using litter size as covariate. Finally all piglets data were analysed using the general linear model (GLM) procedure with the one-way analysis of variance (ANOVA) using piglets status at weaning (survivor or dead) as fixed effect.

RESULTS AND DISCUSSION

Reproduction performance is presented in tables 1 and 2, respectively.

Table 1. Effects of genotype on reproduction performance (means±sem)

	AL (n=10)	LL (n=10)	SL
Gestation length (days)	110.7 ± 0.4	115.2 ± 0.4	p < 0.001
Litter size (n)			
Total born	8.6 ± 0.86	11.7 ± 0.86	p < 0.05
Born alive	8.2 ± 0.76	11.2 ± 0.76	p < 0.05
Farrowing length (min)	134 ± 21	173 ± 21	ns

AL – alentejano breed; LL – Large-White x Landrace; SL – significance level; ns – no significant

There were marked effects of genotype on reproduction performance. As previously reported by Nunes (1993) the AL sows had shorter gestation length than the LL sows. Also prolificacy was lower than in LL sows. However prolificacy of AL sows is in the range of those reported by Marques (2002) and Charneca (2001). Mortality rate between birth and weaning averaged 24.3% for the AL piglets that was higher than the 14.3% found in LL piglets. In both genotypes most of the losses occurred within 3 days after farrowing (85% and 75% for AL and LL piglets, respectively). These observations agree well with those reported by Marques (2002) and Le Dividich and Rooke (2006).

Table 2. Effects of genotype on litter weight, litter gain (birth-24h) and colostrum production of the sows (means±sem)

	AL (n=10)	LL (n=10)	SL
Non adjusted data			
Live born litter weight (g)	9087 ± 839	14893 ± 839	p < 0.001
Litter weight gain 0-24h (g)	533 ± 189	1263 ± 189	p = 0.014
Colostrum Production (g)*	1946 ± 282	3627 ± 282	p < 0.001
Adjusted for live born litter size			
Live born litter weight (g)	10388 ± 526	13574 ± 526	p < 0.001
Litter weight gain 0-24h (g)**	490 ± 193	1306 ± 193	p < 0.05
Colostrum production (g)*	2100 ± 279	3474 ± 279	p < 0.01
Adjusted for live born litter weight			
Litter weight at 24 hours (g)**	12615 ± 192	13142 ± 192	ns
Litter weight gain 0-24h (g)**	634 ± 192	1161 ± 192	ns
Colostrum Production (g)*	2472 ± 245	3102 ± 245	ns

* Calculated by summing individual consumptions of littermates. ** all piglets, survivors or dead
AL – alentejano breed; LL – Large-White x Landrace; SL – significance level; ns – no significant

Litter weight gain within the first 24 hours is a good marker for colostrum production of the sows (Le Dividich *et al*., 2004). It was higher (p = 0.014) in LL than in Al sows suggesting that LL sows are better producers of colostrum. Further, as mentioned by Le Dividich *et al*. (2004) this litter weight gain is very variable, with a CV % approximating 63% in both genotypes.

As expected from the above, colostrum production is higher (p < 0.001) in the LL genotype. Covariant analysis indicates that colostrum production of the sows is dependent on the weight of the litter at birth, but not on the litter size. It could be hypothesised that heavy piglets at birth are also more vigorous and hence more able to extract colostrum from the udder as suggested by Van der Steen and de Groot (1992). The fact that litter weight gain was independent of litter size agrees with the previous findings of Devillers *et al.* (2005). It follows that, within a litter, the colostrum available per piglet decreases with the increase in litter size, which could explain, at least partly, the positive relationship usually reported between litter size and mortality.

The effects of genotype on colostrum and milk composition are presented in table 3. The effects of genotype, time and the interactive effects between genotype and time are also presented. For clarity mean standard errors are not presented.

Table 3. Effects of genotype and time on the composition of colostrum and milk.

		COLOSTRUM						MILK	EFFECTS		
	G	0h	3h	6h	12h	24h	36h	15d	G	T	GxT
Dry Matter (%)	AL	27.3^{a}	27.5^{a}	26.0^{a}	21.1^{b}	20.4^{b}	21.0^{b}	19.0^{b}	ns	***	*
	LL	24.9^{a}	24.4^{a}	23.7^{ab}	22.8^{ab}	19.9^{b}	21.0^{ab}	19.8^{b}			
Crude Protein (%)	AL	17.9^{a}	16.7^{a}	15.0^{a}	10.3^{b}	8.1^{bc}	7.9^{bc}	5.0^{c}	ns	***	ns
	LL	16.6^{a}	15.3^{ab}	13.4^{bc}	10.6^{c}	7.1^{d}	7.0^{d}	4.8^{d}			
Total Lipids (%)	AL	5.4^{a}	6.3^{ab}	6.6^{ab}	6.1^{ab}	7.3^{ab}	8.3^{bc}	8.5^{c}	ns	***	ns
	LL	4.6^{a}	5.4^{ac}	6.8^{abc}	7.8^{b}	7.7^{bc}	9.1^{b}	9.0^{b}			
Lactose (%)	AL	3.0^{a}	-	3.3^{a}	3.4^{a}	5.9^{b}	4.1^{c}	5.4^{b}	ns	***	ns
	LL	2.8^{a}	-	3.5^{a}	3.8^{a}	5.3^{b}	3.9^{a}	5.6^{b}			

G – Genotype; AL – Alentejano sows; LL – LW x LR sows; T, time. *; $p < 0.05$; ***; $p < 0.001$; ns – not significant. Within a line means with different letters indicate significant differences ($p < 0.05$)

Changes in colostrum composition over the first 36h are shown in table 3. There was no significant effect of genotype on colostrum or milk composition. Dry matter, lactose, fat and protein concentrations remained practically unchanged for 6h after the birth of the first piglet. Thereafter, there was a gradual decrease in protein content and a gradual increase in fat and lactose. Overally, the composition of colostrum over the first 36 hours after the onset of parturition was largely similar to those reported previously (Klobasa *et al*., 1987; Le Dividich *et al*., 2004).

Data on piglets' birth weight, weight gain from birth to 24h of age and colostrum consumption of AL and LL piglets are presented in table 4.

Table 4. Piglets' birth weight, weight gain and colostrum intake (means ± sem)

	AL	LL	SL
Birth weight (BW) (g)	1106 ± 27 (n=82)	1330 ± 23 (n=112)	$p < 0.001$
Weight gain (birth-24h) (g)	65 ± 10 (n=82)	113 ± 9 (n=112)	$p < 0.001$
Colostrum intake (g)	266 ± 14 (n=73)	336 ± 12 (n=108)	$p < 0.001$

n, number of piglets; AL, alentejano breed piglets; LL, crossbreed piglets; SL, significance level

At birth AL piglets were lighter ($p < 0.001$) than LL piglets. Also their weight gain and colostrum intake in the first 24h were inferior ($p < 0.001$) than those observed in LL piglets. This effect of birth weight on weight gain from birth to 24h and colostrum consumption is in agreement with the findings of Le Dividich *et al*. (2004) and Devillers *et al.* (2005).

Lower colostrum intake in Al piglets can be related both to the lower ability of AL sows to produce colostrum and the lower birth weight of the piglets (Le Dividich *et al*. 2004). However, when expressed per Kg birth weight there was no significant effect of genotype on colostrum consumption of the piglets. In both genotypes, the birth weight is positively and significantly correlated with the weight gain (r^2=0.53, $p < 0.001$) and colostrum consumption during the first day of life ($r^2 = 0.59$, $p < 0.001$). When expressed in g / Kg birth weight, the relations remain significant for weight gain ($r^2 = 0.43$, $p < 0.001$) and colostrum consumption ($r^2 = 0.18$, $p < 0.05$), in accordance with the observations of Le Dividich *et al*. (2004) and Devillers *et al.* (2005).

Characteristics of piglets dying after birth are shown in table 5.

Table 5. Characteristics of piglets dying after birth compared to those of survivors. (means ± sem).

	AL PIGLETS		SL
	dead	**survivors**	
Number of piglets	20	62	
Birth weight (BW) (g)	982 ± 42	1146 ± 24	p < 0.001
Age of death (days)	2.2 ± 0.6	-	-
Weight gain (birth-24h) (g)	0 ± 14	86 ± 8	p < 0.001
Body weight at death, g	1022 ± 73	-	
	LL PIGLETS		
	dead	**survivors**	
Number of piglets	16	96	
Birth weight (BW) (g)	1013 ± 62	1383 ± 25	p < 0.001
Age of death (days)	3.2 ± 0.9	-	-
Weight gain (birth-24h) (g)	15 ± 24	129 ±10	p < 0.001
Body weight at death, g	1014 ± 80	-	

SL, significance level

In both genotypes, piglets dying during the nursing period have in common to be lighter ($p < 0.001$) at birth and to gain much less weight ($p < 0.001$) during the first 24 h than survivors. Estimation of colostrum consumption of these piglets is not, however, possible because many of them died before 24 h of age. However, their body weight gain from birth to 24 h of age or to death time suggest that these piglets have consumed much less colostrum and (or) milk than survivors. It could be hypothesised that survival of these piglets is doubly impaired by a low birth weight and a lower colostrum and (or) milk consumption which both reduce the vigour of these piglets and their ability to compete at the udder. However, a larger data set is required to determine the respective role of birth weight and colostrum consumption on post-natal mortality.

CONCLUSIONS

Data of this study indicate colostrum and milk composition of Alentejano and modern sows is similar whereas AL sows produce less colostrum. In both genotypes, production of colostrum is dependent on the weight of the litter but not on the litter size, while within each genotype, consumption of colostrum is dependent on the piglet birth weight. In both genotypes, piglets that died before weaning were lighter at birth and had consumed less colostrum or (and) milk than survivors. Further studies are necessary to determine the effects of genotype on the immune quality of colostrum.

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ORGANIC BREEDING HERD: MANAGERIAL DIFFICULTIES IN ORDER TO SATISFY THE REQUIREMENTS OF THE LACTATION SOWS

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SUMMARY- Organic breeding is expanded rapidly in Europe. The actual development can be attributed to an increased consumer interest in organic products throughout Europe It is important to evaluate the suitability of commercial breeds for organic environments analysing chemical clinical parameters to evidence eventual problems due to managerial mistakes or to difficult of adaptation to the organic breeding

Key words: organic breeding, lactation sow, biochemistry parameters

INTRODUCTION

Organic breeding in Europe is continually increased in last years. The actual development can be attributed to an increased consumer interest in organic products (Barton, 2002).
In the European countries, the EEC-Regulation No 1804/1999 has been passed and become law in August 2000. This law provides a standard that involves the right to label food as organic. It includes specifications for housing conditions, animal nutrition, and animal breeding, as well as animal care, disease prevention, and veterinary treatment.

In pig production, it is virtually impossible to find animals that have been bred specifically for organic production systems (Bodelling *et al.*, 2003). Therefore, most farmers use animals from conventional breeding companies. In the organic breeding the feed intends to ensure quality production rather than to maximize production. Feed materials of agricultural origin can be used only if they are produced or prepared without the use of chemical solvents. This implies that e.g. soybean meal, the most common protein source in animal nutrition, cannot be used in organic feed.Antibiotics, coccidiostats, medical substances, growth promoters, or any other substance intended to stimulate growth or production are not allowed in animal feeding No feed components may have been produced with the use of genetically modified organisms. Vitamins authorised for conventional animal production under Directive 70/524/EEC should be derived from raw materials occurring naturally in foodstuffs. Synthetic vitamins identical to natural vitamin can, however, be used for monogastric animals. Livestock must be fed on organically produced feeding stuffs, preferably from the farm itself.
The aim of this work is to evaluate the suitability of these animals for organic breeding analysing chemical clinical parameters to evidence, eventual problems due to managerial mistakes or to difficult of adaptation to the organic breeding.

MATERIAL AND METHODS

We sampled 20 lactation sows in an organic breeding. The herd, located on an hilly area, has a population of 30 productive commercial hybrid sows. Every department is working on "all in/all out" basis, apart from the breeding stock.

All samples were collected in the morning hours before feed administration. Blood samples were taken by jugular venepuncture into a litio/eparina vacutainer. Plasma was collected after refrigerate centrifugation (3000 rpm, 15 minutes, 4°C). The samples were stored at –20°C until are tested. The plasma of these animals was analyzed using an Hitachi 704 to determinate the follow biochemistry parameters: Total Protein, Albumin, Glucose, Urea nitrogen (BUN), Creatinine, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Gamma glutamyl transferase (γ GT), Calcium and Phosphorus.

RESULTS AND DISCUSSION

Test results are summarized in Table1. Mean values obtained in our samples are in the normal ranges for almost all parameters. There are statistically significative differences between references value (Kaneko *et al.*, 1997) and results obtained only for three parameters: Total protein, Albumin, Urea nitrogen.

Total proteins are directly influenced by nutritional level (Meyer and Harvey, 1998). Low level of total proteins can be due to an increased loss (enteropathy, nephrosis), malabsorpion, decrease production (chronic hepatic disease) or malnutrition.
Albumin production is solely dependent on the liver (Meyer and Harvey, 1998) and an adequate nutrition. A reduced serum concentration is associated with chronic hepatic insufficiency, increased loss in the urine or gastrointestinal tract, malnutrition, and down regulation of albumin production secondary to hyperglobulinemia.

Protein is the major source of ammonium for urea and the rate of urea formation depends on the rate of protein catabolism. The ingestion of decreased quantities of high- quality proteins results in decreased BUN. There is a decreased production of urea for severe hepatic disease, malnutrition, malabsorbition .

CONCLUSIONS

These results are probably due to an inadequate feeding. This kind of feeding does not satisfy the metabolic needs of these animals that are not specifically bred for organic production system.

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Table 1: Reference values and results of the samples

	P.T. g/l	Albumin g/l	Urea (BUN) mmol/l	Glicemia mmol/l	Creatinine μmol/l	ALT U/l	AST U/l	γGT U/l	Ca mmol/l	P mmol/l
Reference values (Kaneko)	65- 90	30- 40	2.5- 9	2.9- 5.9	140- 200	20-60	36-880	10-60	1.9-2.8	1.5-2.2
Mean of Resultes	53.2	24	1.4	5.8	142	59	69	26	2.6	2.1
P	<0.001	<0.001	<0.001							

ANIMAL WELFARE AND PRODUCTION IN THE IBERIAN PIG

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SUMMARY - The Iberian pig has been asociated traditionally with outdoors production systems. The morphologycal, etologycal and physiological characteristics of Iberian pig are adapted to take full advantage of mediterranean ecosystem from the SouthWest of Spain. The economic peak of Iberian pig sector have prompted more intensification in the farms. Now most of the farms find alternative production systems compatibles with the natural environment and animale welfare. In this research we have been performed to measure the productivity in three different rearing systems: outdoor system, traditional system and intensive system with farrowing crate. Our subject were thirty six Iberian sows, divide in three sets of twelve animals each one. A total of four suckilng periods data were collected, for two years to value different productive index: piglet born alive, piglet weaned alive, percentage perinatal survival percentage, suckilng period survival percentage and daily average gain. The intensive rearing system showed the higher percentage of perinatal survival (87,7%).The outdoor system offered the best lactation survival rate (79 %). The dairy average gain for piglets was higher in outdoor system. In conclusion, the most productive system was the intensive system with a higher farrows medium weight.

Key words: Iberian pigs; rearing systems; productive parameters; animal welfare.

INTRODUCTION

Traditionally, the Iberian pig has been related to extensive or semi-extensive productive systems, going so far as to form an almost perfect balance with ecosystems like the dehesa. This relation is not occasional, since the morphologic, ethological and physiological characteristics allow this breed (Laguna Sanz, 1998) to fully optimize the resources that this ecosystem offers. Nowadays the Iberian pig keeps on supporting a very important link with the dehesa, but due to the tendency and the evolution of the porcine sector, “productive variables” are introduced in each phase of production, from breeding to fattening.

From middle of last century the Spanish porcine sector has experienced an important structural evolution, and precocious breeds have been introduced (Pietrain, Landrance, Large White, etc.) the production has passed from a local to an intensive production that currently represents 90 % of the livestock pig census (Dieguez Garbayo, 1992). The Iberian pig has not been an exception and has over the last years taken part in the modifications of the sector.

Fame of the Iberian pig derived products from have experienced over the last years, thanks to their quality, has been translated into a increase in the census and the number of specific farms, going from a traditional system strongly linked to the environment, to others with high intensification and fragmentation of the production stages (Gomez – Nieves and Robina, 2005).
The intensive systems of production used in many farms, have been developed according to the breed standards of the precocious porcine breeds (Edwards, 2001). It would be necessary that the Iberian pig farms would develop themselves but would also take care of the proper characteristics of this breed. Nevertheless, the reality is different and the final result is a transposition of the systems and handling used for precocious breeds, with minimal adaptations for some animals which have

been selected for centuries, for their wildness and adaptation to be bred in outdoor production, which is determinant of their quality.

At a moment when precocious pig farms is looking for compatible and alternatives that are compatible with the environment and copying the outdoor production earlier discarded due to its high costs, the Iberian livestock sector is going towards a major intensification leaving precisely this extensive production (Ambrogi, 2001), which has provided the privileged situation that it occupies.

We cannot forget the tendencies of the Common Agricultural Politics of the European Union, that are based on productions that must be environmental-friendly and respectful with the animal welfare. Nowadays the conditions of animal welfare are particularly important in the legislation. This interest has been enhanced in the new Common Agricultural Politics by the needed condition to fulfil animal welfare in order to get a subsidy.

Departing from this point, it is necessary to bear in mind that all the stockbreeders farms and in particular porcine ones, are conceived to obtain the maximum benefit from the productive animals. It is therefore necessary to find the balance between a profitable livestock and some conditions for the animals that will allow, the expression of all its genetic potential (Aparicio and Vargas, 2006).

MATERIAL AND METHODS

In this work we try to establish indicators of animal welfare for the Iberian pig, to value three representative systems. The rearing stage needs a big attention, because errors in the approach, handling or health can have critical consequences on the profitability of the exploitation (Prieto and Roy, 2003).

We study the productivity of 36 pure Iberian sows, in “Valdesequera” farm, which belongs to the Service of Research and development of Junta de Extremadura. Three lots of 12 sows were done after a in group gestation and they were hosted in three rearing systems with different facilities and handling.

Intensive system: with farrowing crates and some heating badge for the piglets; the feeding is automatically, automatic water troughs and natural ventilation and lighting by adjustable windows and the typical reproductive handling. The feeding of the piglets is concentrated food ad libitum by means of hoppers placed in every cubicle.

Traditional rearing system: with individual pens, concrete soil and straw bed. The piglets have access to an exterior and individual pen with automatic watering devices and hopper for feeding the piglets with concentrated food from 21 days. The sows feed the piglets during the night and 2 hours from 12 pm to 2 pm for twenty-one days after farrowing.

Outdoor system: in a fence of 1,5 hectares, in which there are 12 iron campings. The feeding of the sows is realized in hopper and they can access to an artificial lagoon as a drinking point. The camping have a barrier to prevent piglets that going out until 21 days. In this moment they have access to the whole fence. The drinking point of the piglets is the same artificial lagoon and the feeding is concentrated food in a common hopper.

The productive samples: individual weighing at birth, weekly weighing for 5 weeks up to the weaning, total number of piglets born alive, number of piglets born alive and dead and number of piglets weaned per litter.

The data has been taken in four consecutive suckling periods: autumn 2004, spring 2005, autumn 2005 and spring 2006.

From this data, we could obtain a series of necessary productive indexes to compare the three systems used in the study: perinatal survival (nv x 100 / nt) represented by the born alive (nv) total born (nt); survival during the lactation (vd x 100 / nv) represented by the weaned piglets (vd) with regard to the total born alive number (nv); daily average gain (GMD); litter average weight at weaning (PCD); litter size at weaning (TC).

RESULTS

The perinatal survival (Table 1) shows in percentage terms the entire number of piglets that are born in every litter with the number of piglets that are born alive or have overcome the first hours of life. Therefore, a high postnatal survival indicates the efficacy of every system just a minor number of crushed piglets and a better access for piglets to the nipples of the sows in the first hours of life. When analyzing the results, we find out that the intensive system (87,8 %) is the one that presents better results, compared to traditional system (80,1 %) and outdoor (81,9 %) that show similar values (Graph 1). This result is explained because the farrowing crates of the intensive system are precisely designed to avoid the two mentioned problems.

Table 1. - Percentages of perinatal survival and during the lactation period in three productive systems.

	postnatal S.	S. during lactation
Intensive	**87,8**	76,0
Tradicional	**80,1**	70,7
Outdoor	81,9	79,0

The survival during lactation (Table 1) compares by means of percentage the average size of the litter. With this index we can measure the adaptation of the piglets to the conditions of every rearing system, mainly determined by the appearance of infectious sanitary problems. In that case the most effective system, as more piglets come to the weaning, is the outdoor (79 %), secondly the intensive system (76 %) and the system in which less piglets come to the weaning is the traditional system (70,7 %). The outdoor is the system with a minor presence of sanitary problems for piglets (Graph 2).

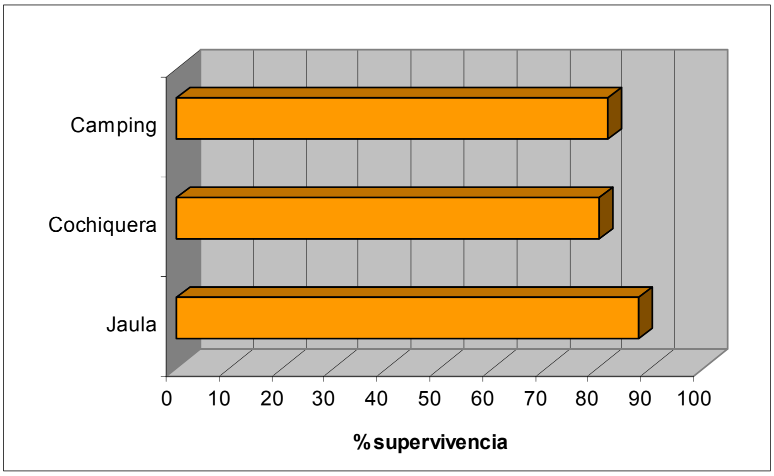

Figure 1. - Percentages of perinatal survival in three productive systems.

The other indexes regarding the weight increases of the piglets along the lactation period. We started by comparing daily average gain (Table 2; Graph 3), that indicates the growing rhythm of the piglets. The outdoor system is the one that presents the best results of growth of the piglets, while in intensive and in traditional system we do not find differences. Nevertheless, to be able to compare these results it is necessary to bear in mind the size of the litters in the weaning, since the above mentioned size determines the distribution of resources of the sows for a bigger number of piglets.

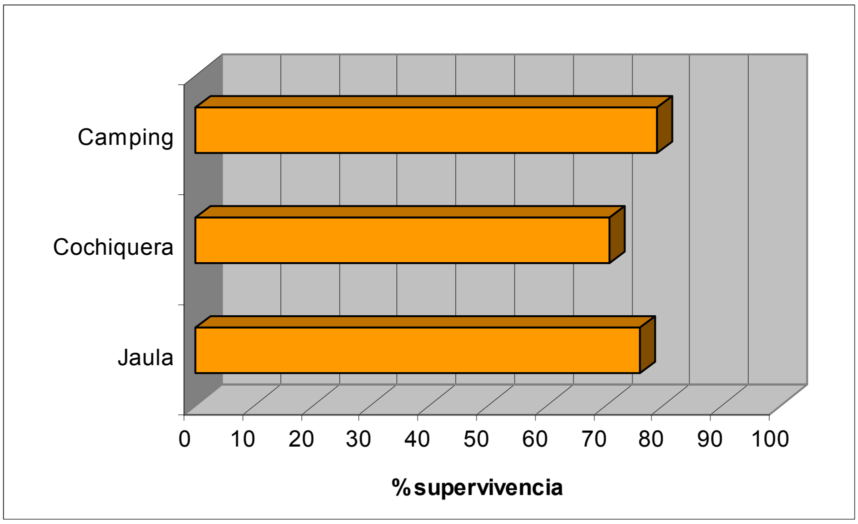

Figure 2. - Percentages of survival during the weaning in three productive systems.

A way of comparing the growth results is to calculate the middleweight of the litters at the weaning for each system, multiplying the average size of the litter to the weaning (Table 2) by the increase of weight of the piglets during the lactation period, so that we can make corrections according to the litter size. Once corrected according to the litter size, we find out that the intensive cages system presents a major middleweight of the litter to the weaning (41,617 kilograms) and, therefore, the most productive, even if it is the system in which GMD and PCD are minor, the result is compensated thanks to the biggest size of the litters (Table 2). Among the results of the traditional system (34,564 kilograms) and outdoor (33,975 kilograms) there is not a significant difference.

Table 2. - Daily average gain (GMD), middleweight of the litter until weaning (PCD) and size of the litter to the weaning (TC), in three productive systems.

	CAGE			HOG HOUSE			ARC		
	GMD	**PCD**	**TC**	**GMD**	**PCD**	**TC**	**GMD**	**PCD**	**TC**
1st period	196,1	43320,9	7,3	201,1	27041,3	4,5	230,9	42021,0	6,0
2nd period	188,8	38935,2	6,6	194,6	43099,0	7,0	231,3	37231,0	5,0
3rd period	199,4	42964,4	6,8	195,0	36870,0	6,3	235,1	26750,5	3,8
4th period	196,9	41464,7	6,7	177,3	31248,7	5,4	233,6	29898,5	4,6
Average	195,3	41671,3	6,8	192,0	34564,8	5,8	232,7	33975,2	4,8

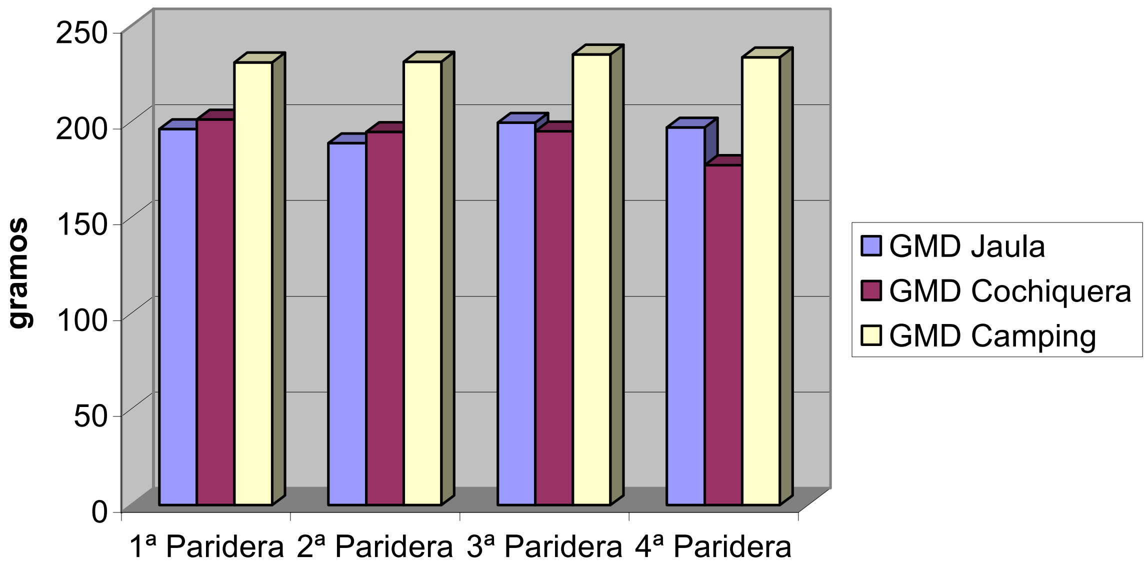

Figure 3. - Daily average gain (in grams) in three animal breeding systems along four farrowing pens

CONCLUSIONS

The intensive system is the most productive of the three under study, since all its indexes are higher than the traditional and outdoor systems. Nevertheless, it is necessary to bear in mind that all the considered indexes are influenced by the first one, the postnatal survival, and these bad results in this stage are going to be accumulated although, as we have seen, the rest of indexes are better in outdoor than in intensive system.

Certainly the ideal thing would be to reach a balance between a profitable production of the exploitations and some conditions that can assure animals' welfare, bearing in mind the peculiarities of every breed.

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ANNEX . Pictures

Photo 1. Traditional system.

Photo: Group of Investigation in Animal Welfare of the Uex.

Photo 2. Intensive system

Photo: Group of Investigation in Animal Welfare of the Uex.

Photo 3. Outdoor system

Photo: Group of Investigation in Animal Welfare of the Uex.

INFLUENCE OF ENVIRONMENTAL CONDITIONS IN IBERIAN PIG REARING SYSTEMS

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SUMMARY – The intensive systems used in the production of iberian pig can supose a lost of welfare. For this fact, is necesary to know the influence that the eviroment conditions exert in the iberian pig rearing.For fix the influence of the temperature and the humidity, we took datas of four animalbirth time. To make the study we used a total of 36 breedings of iberian pig, divided in three sets of 12 animals each one. Each set of animals had a diferents rearing sistem: intensive with crates, chochiquera with a traditional use and camping sistem. After analice the results, we checked that the conditions of temperature and humidity influence depending of the animalbirth time. The SUPERVIVENCIA PERINATAL rate in the outom animaldirth time (85,9%) are more tahn spring time (80,7%). Meanwhile that the SUPERVIVENCIA during the spring suklig time (79,2%) is more than outom sukling time (71,3%). Also we check that the sistem of camping is the best one in the adaptiton to animalbirth time, because it present less productive variations between both animalbirth time.

Key Words: Iberian pig; enviromental parameters; production; animal welfare.

INTRODUCTION

During the last decades, Iberian pig sector has experienced an extraordinary impulse, which produced an increase either in reproductive census or in the number of exploitations: from 97.858 heads in 1995, to 222.023 in 2005 (MAPA, 2006). The recovery of this Iberian pig census started in 1986, moment when finished a crisis that could have jeopardized this animal as a livestock breed. The constant demand of Iberian pig products is justified by the economic progress of the consumers and by the elimination of sanitary obstacles that were preventing the opening to exterior markets (Varga and Aparicio, 2000).

The proper characteristics of this breed have helped to optimize the resources of the dehesa ecosystem present in the south-west of Spain. The image of the Iberian pig is link to a natural and ecological production. Nowadays, this type of productions, traditionally connected to the environment, has passed to other productive variables more intensive.

The transformation of the Spanish pig farms, initiated in the sixties, supposed the introduction of a noticeable intensification of the systems, using facilities and methods proper for precocious breeds. In this aspect, the Iberian breed has not been an exception, and has the modifications carried out over the last years too.

The adaptation of the intensive systems to the productions of the Iberian pig has been realized without bearing in mind the proper physiological and ethological characteristics of this breed. This can imply a serious loss in animals' welfare, and consequently a decrease of the productivity in the pig farm. As very few studies have been done on this matter, we need to study the conditions of welfare in the production of this breed. We started analyzing one of the critical moments of the production of this animal (the breeding period), although this work is part of wider other, in which there will be analyzed the rest of the productive phases.

To value animals' welfare at breeding period, it is necessary to study the environmental conditions to which the sows are submitted in their facilities, as they can often generate stress in the animals. That's why it is important to know the influence that such factors as temperature and humidity can have in the different productive systems used for this breed. This is how we will be able to keep animals in a range of temperatures that will help them to feel comfortable enough as to develop a normal activity (Quiles and Hevia 2004). It is therefore necessary to find the balance between a profitable exploitations and some conditions for the animals that allow the expression of all its genetic potential (Aparicio and Vargas, 2006)

Our biggest determination is that these works can be used as an orientation for the Iberian pig farms, sticking to the scientists' tendency, which over the last years has developed a porcine breed, which coped with very important adversities along the history, but that has finally received the fully deserved recognition (Vargas and Aparicio, 2000).

MATERIAL AND METHODS

The experimental development was carried out in "Valdesequera" farm, which belongs to the Agriculture Investigation Department of the Junta of Extremadura. We were used for the study 36 sows. The data was colleted during four breeding period, both from the reproductive animals and their litters. These animals were split into 3 lots of 12 sows, distributed in three representative rearing systems: intensive with crates (Slat), traditional handling (Cochiquera) and out doors system (Camping).

The intensive systems are characterized by individual crates of sows and a natural ventilation and lighting system. The feeding of the animals is automatically done with individual dispensers, which allows a constant control of the sows' intake.

Another system used in this experience is the cochiquera system, traditionally used in Iberian pig breeding. This system is provided with approximately 3 m^2 for each sows, where a straw bed is set to let the reproductive animals make their nest. It also contributes to the thermo-comfort of the piglets. The premises are provided with exterior corrals for the piglets and they have access through a door. There is a 1 hectare fence that surrounds the premises so that the reproductive animals can enjoy it and use it. Animals are fed in this place and the automatic watering devices are placed there as well.

The last used system is out doors. It requires minor infrastructures. In a 1,6-hectares fence, 12 metallic huts are directly placed on the soil and the reproductive animals have a free access. A straw bed is placed inside to allow the elaboration of the nest and avoid hypothermia at piglet. In the fence there is a lagoon that serves as drinking station for the animals and a special trough for piglets, to which they have access from 21 days of life on.

For the productive results of the animals, the following indexes were taken into account: perinatal survival (nv x 100 / nt) represented by the born alive number (nv) with regard to total born number (nt); suckling time survival (vd x 100 / nv) represented by the weaned piglets (vd) with regard to the total born alive number (nt); daily average gain (GMD); litter average weight (PCD); weaning litter size (TC).

Concerning the influence exercised by environmental factors in the productive data, the environmental characteristics were analysed according to breeding period done in spring or in autumn. The records of temperatures and humidity found in each productive system were done by electronic devices provided with specific sensors for the chosen parameters

RESULTS

The results obtained for the reproductive animals (Table and graph 1) show significant differences between the indexes of survival according to the breeding period. This data determines that the perinatal survival, (the proportion of born alive piglets compared to the total born number is significantly major in autumn (85,9 %), than in spring (80,7 %); while the suckling survival is significantly higher in spring (79,2 %) than in autumn(71,3 %).

The daily average profit of piglets according to the breeding period (Table and graph 2), shows very similar values. It might therefore be deduced that the breeding period does not influence in the litter average weight to the weaning. Nevertheless, if we analyze these results bearing in mind the rearing system, the results show that the intensive systems and traditional system are more affected by the breeding period, as the litter average weight diminishes in autumn; 3 kilograms in the case of intensive system and 5 kilograms in traditional system. On the contrary, the out doors system is the one that turns out to be less affected by seasons (there are not significant differences between autumn and spring).

So we can see that at the beginning of autumn, the temperature (25ºC), is closer to the thermo-comfort stage for piglets. This helps reducing the number of deaths by crushing and hypothermia, increasing survival numbers then. As breeding period goes on, the temperature tends to decrease, reaching 18ºC at the end of the suckling period, which is farther from the ideal temperature for piglets and leads to a reduction of survival numbers.

In spring time the opposite happens. The minor postnatal survival of piglets might be related to a lower temperature at the beginning of breeding period (18ºC), far from the thermo-comfort stage and with an increasing tendency of the above mentioned temperature, which favours the survival during the whole lactation period with values next to 30º C.

Joined to the temperature there is another important element: humidity, which normally diminishes when the temperature increases. In temperatures between 20ºC to 30ºC, the relative moisture does not have much influence, but the negative effects are accentuated by temperatures superior to 30ºC, as well as with low temperatures, a high environmental moisture can provoke important caloric losses in pigs (Granier *et to.*, 1996).

The values of moisture obtained in this work would have a minor influence at the beginning of autumn and the end of spring, and this diminishes the possible adverse effect of the high temperatures and favours perinatal survival in autumn and the survival in suckling period in spring. Nevertheless, the moisture is high at the end of autumn and beginning of spring, as temperatures are minor to 20ºC, so the humidity accentuates the cold sensation and negatively affects survival suckling period in autumn and perinatal survival in spring (Graph 3 and 4).

If, furthermore, the productive system is analyzed, it can be observed that these effects are clearer in the intensive systems and, especially, in the traditional system, where the values of survival in suckling period in autumn, are lower (64,4 %). Then the indexes of the out doors system are the ones that better answer to this seasonal change.

Concerning the litters average weights in autumn and spring, the decrease of the survival index during the suckling period can be justified due to the tendency that the temperature values are more distant to the thermo-comfort ones for piglets as breeding period progresses in the case of autumn and the process is the opposite in spring.

CONCLUSIONS

We finally draw two conclusions, which we consider important.

The conditions of temperature and humidity, in the two breeding period (autumn and spring), have an influence on the productivity of sows and scarcely influence on the productivity of piglets.

The out doors system presents major daily oscillations, but in spite of that, it is better adapted to the season aspect of breeding period (autumn and spring) and to temperature and humidity conditions, since productivity varies very little in both farrowing.

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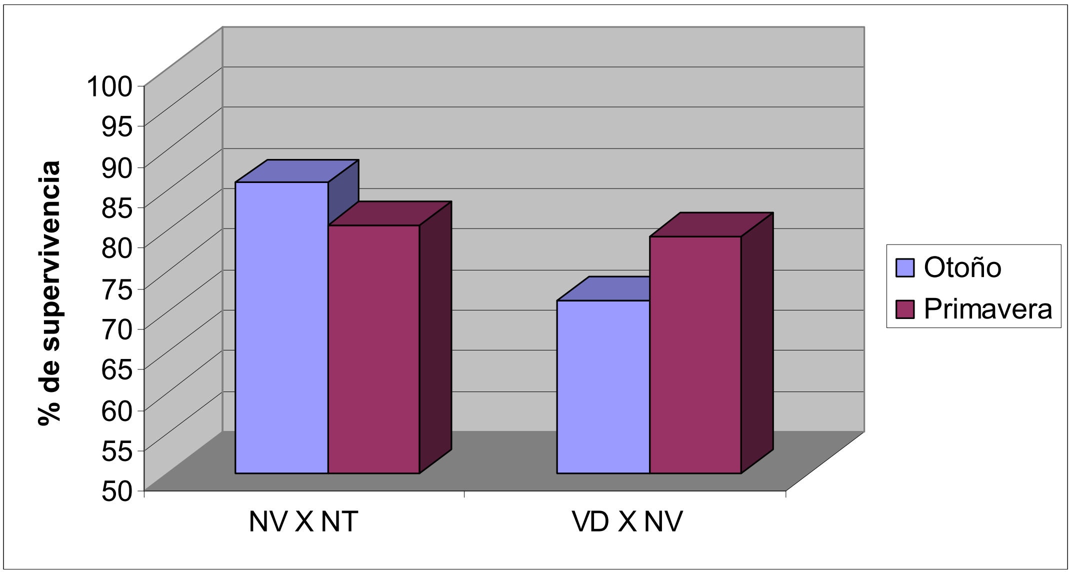
Table 1. - Percentages of perinatal survival (% NV X NT) and suckling period survival (% VD X NV).

	AUTUMN		**SPRING**	
	% NV X NT	**% VD X NV**	**% NV X NT**	**% VD X NV**
Intensive	91,4	72,1	84,1	80,0
Traditional	80,4	64,3	79,9	77,1
Out doors	85,8	77,5	78,0	80,4
Average	85,9	71,3	80,7	79,2

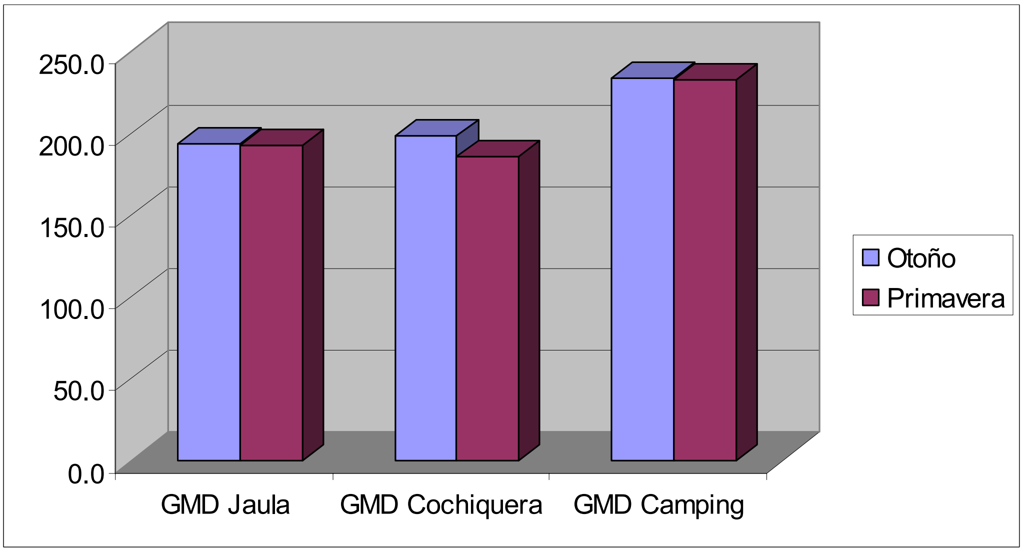
Table 2. Daily average gain of piglets.

	AUTUMN	**SPRING**
GMD intensive	193,3	192,8
GMD tradicional	198,0	186,0
GMD out doors	233,0	232,4
Average	208,1	203,7

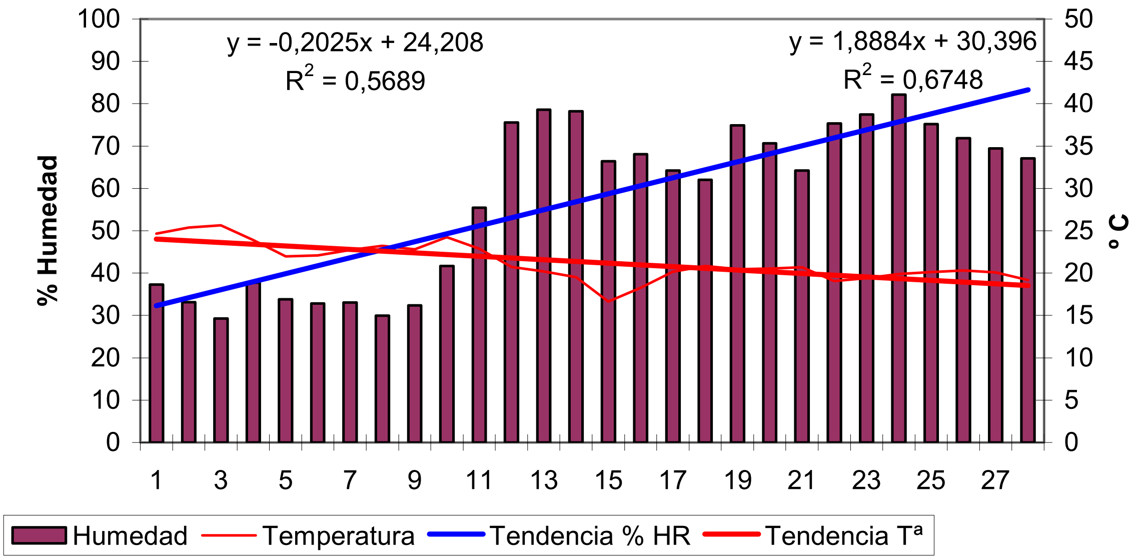
Figure 1. Comparison of the percentages of perinatal survival (% NV X NT) and suckling period survival (% VD X NV). Where NV = born alive, NT = total born, VD = weaned alive



Graph 2. Average profit daily (GMD) for three productive systems in every farrowing period



Graph 3. - Evolution of the temperature and humidity during the suckling period in autumn



Graph 4. - Evolution of the temperature and humidity during the suckling period in spring.

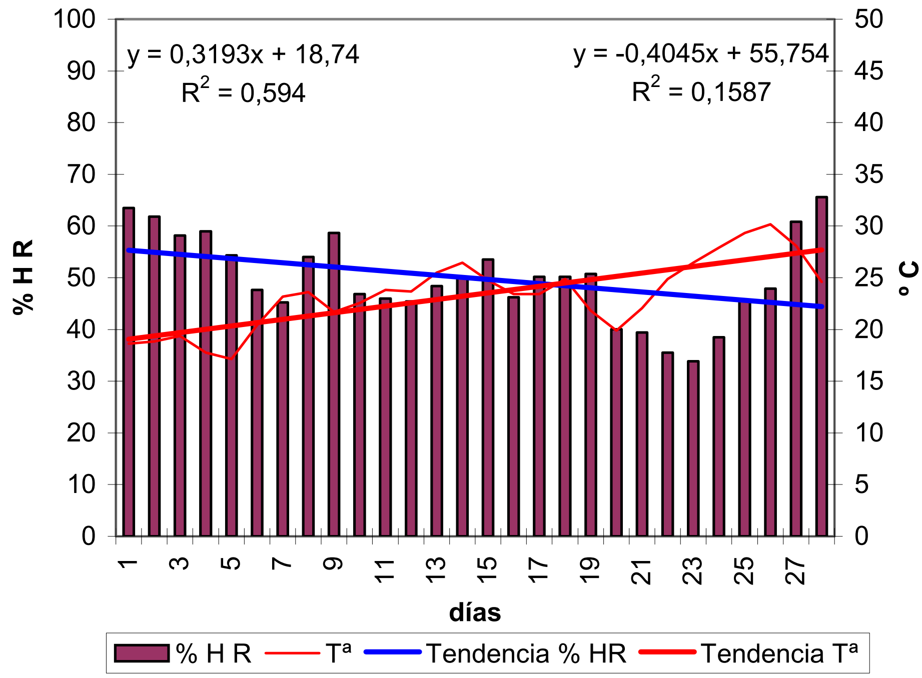


Photo 1. Iberian piglets in intensive system. Author: Group of investigation in animal well-being, UNEX.

Photo 2. - Iberian piglets in traditional system (Cochiquera). Author: Group of investigation in animal well-being, UNEX.

Photo 3. - Iberian piglets in out doors system (Camping). Author: Group of investigation in animal well-being, UNEX.

EVOLUTION OF THE COMPOSITION IN FATTY ACIDS OF THE DORSAL SUBCUTANEOUS ADIPOSE TISSUE FROM ALENTEJANO PIGS FED ON PASTURE AND ACORNS

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SUMMARY - The present work studied the evolution of fatty acid composition in the dorsal subcutaneous fat of free-range Alentejano pigs fed on pasture and acorns. Twenty pigs with a body weight (BW) of ~90 kg were placed in a 2 ha pasture (*Lolium westerwoldium* and *Trifolium incarnatum*) and fed daily with 4 kg of acorns. Animals were submitted to biopsies near tail insertion with ~90, ~100, and at slaughter, ~110 kg BW. The fat samples obtained were analysed by GC/FID to determine the fatty acid composition. An increase of only 20 kg body mass based on pasture and acorn consumption led to: (i) carcasses with C18:1 content similar to the minimal value stipulated by market demands for Alentejano pigs bred in free-range conditions; and (ii) contents of C16:0 and C18:0 were within the limits for the carcass-quality assessment. Nevertheless, the contents in C18:2 remained slightly above the 9.8% limit.

Key Words: Alentejano pig; acorn; grass; adipose tissue; fatty acids.

INTRODUCTION

The Alentejano (AL) pig is traditionally reared in extensive systems. During growth, pigs are fed fresh pasture in the spring, stubble in the summer, and by-products from different crops during these two seasons. From October to late February, AL pigs are fattened in "montanheira" with acorn (*Quercus* fruit) and grass. This fattening period lasts usually from about 70-90 to 140-160 kg body weight (BW) (Almeida *et al*., 1993). The acorn is very palatable for the pig, which removes the hull to ingest only the kernel. Voluntary consumption of acorn has been estimated to vary from 6-7 kg in pigs of 50-70 kg to 8 kg in pigs of 80-90 kg BW. At 100 kg BW, this breed consumes about 9 kg of acorns (Laguna Sanz, 1998).

Acorn consumption favours fat deposition and reduces protein accretion (Aguilera and Nieto, 2003). Comparatively with other breeds, the AL pig shows a high lipogenic activity and lipids are deposited mainly in the subcutaneous backfat. The proportion of fatty cuts can attain more than 50% of the carcass weight and the backfat thickness at the last rib level can reach 60 mm at 120 kg BW (Almeida *et al*., 1993; Neves *et al*., 2001). This kind of carcass is considered ideal for the manufacture of dry cured products. In fact, the manufacture industry (cured hams, forelegs and sausages) requires pigs reared in traditional extensive systems, slaughtered at 140-160 kg BW and with 18-24 months of age (De Pedro Sanz and Olmo, 2000). Pigs grown on acorn and grass consumption present chemical, biochemical, and physical changes in the adipose tissue, mainly due to an increase on the lipid content and on the deposition of oleic acid (C18:1), whose concentration on acorn is about 63% (Almeida *et al*., 1993; Neves *et al*., 1994). These changes may determine the adipose tissue global quality (firmness or softness, colour or oxidation sensibility) (Lebret and Mourot, 1998).

The C18:1 is the most abundant fatty acid in AL pig fat depots and has an important role in meat chemical and physical characteristics, mainly in adipose tissue. When pigs are fattened on acorns, the amount of C18:1 in their fat depots can reach ~60% (Almeida *et al*., 1993; Neves *et al*., 1994) depending on the total body mass gain while fed acorns. Due to this characteristic, the C18:1 proportion was converted in a reference for classification purposes of the source of raw matter from "montanheira" (minimum 54% of C18:1). The criteria also include the proportion of palmitic (C16:0) and stearic (C18:0) acids (minimum 21.3 and 9.8%, respectively), and the proportion of linoleic acid (C18:2) (minimum 9.8%), since an increase on C18:1 proportion could lead to a decrease of other fatty acids. According to Aguilera and Nieto (2003), to match these criteria AL pigs should have at least a total gain of 45 kg BW during the fattening period in "montanheira".

The present experiment was undertaken to study the evolution of the fatty acid composition in the dorsal subcutaneous fat of free-range AL pigs fed on pasture and acorns from 90 to 110 kg BW, a 20 kg total body weight accretion.

MATERIAL AND METHODS

Twenty AL pigs with ~90 kg BW were placed in a 2 ha pasture, based on *Lolium* sp. and *Trifolium incarnatum*. Each pig was fed daily with 4 kg of acorns. At an average BW of 90 and 100 kg, all animals were submitted to biopsies near tail insertion to obtain samples of adipose tissue. Finally, the pigs were slaughtered at ~110 kg BW, and adipose tissue samples were also obtained. All these samples were collected according to the technique used by the Association of Porco Alentejano breeders for control of production system. Fat was melted in a vacuum oven at 40ºC for 30 min, and analysed for the composition in fatty acids by GC/FID. An ANOVA was carried out and the means comparison was made by SNK test. SPSS statistical software was used.

RESULTS AND DISCUSSION

Table 1 shows the results of the evolution in fatty acid composition of the subcutaneous fat at the level of tail insertion between 90 and 110 kg BW.

Table 1. Effect of body weight (BW) on fatty acid composition of subcutaneous adipose tissue from Alentejano pigs (n=20) fed on pasture and acorns (mean ± standard error).

	90 kg BW	100 kg BW	110 kg BW	Sig
C12:0	0.080 ± 0.004^{a}	0.085 ± 0.004^{a}	0.069 ± 0.004^{b}	0.018
C14:0	1.27 ± 0.03^{a}	1.27 ± 1.03^{a}	1.14 ± 0.03^{b}	0.003
C16:0	19.46 ± 0.29^{a}	19.14 ± 0.28^{a}	17.36 ± 0.32^{b}	0.000
C16:1	3.46 ± 0.10^{b}	3.86 ± 0.10^{a}	3.95 ± 0.11^{a}	0.003
C17:0	0.349 ± 0.02	0.32 ± 0.02	0.316 ± 0.02	0.429
C17:1	0.44 ± 0.02^{a}	0.39 ± 0.02^{ab}	0.35 ± 0.02^{b}	0.019
C18:0	7.91 ± 0.16^{a}	7.61 ± 0.16^{ab}	7.25 ± 0.18^{b}	0.029
C18:1	49.62 ± 0.40^{b}	50.51 ± 0.39^{a}	53.53 ± 0.44^{a}	0.000
C18:2	14.11 ± 0.34	13.85 ± 0.33	12.93 ± 0.37	0.062
C18:3	0.80 ± 0.04^{b}	0.77 ± 0.041^{b}	0.86 ± 0.047^{a}	0.015
C20:0	$0.20 \pm .02$	0.19 ± 0.016	0.26 ± 0.02	0.364
C20:1	1.27 ± 0.03	1.13 ± 0.03	1.28 ± 0.04	0.060
C20:2	0.82 ± 0.03^{a}	0.67 ± 0.027^{b}	0.57 ± 0.03^{c}	0.000
C20:4	0.21 ± 0.01^{a}	0.20 ± 0.01^{a}	0.14 ± 0.01^{b}	0.000
$\sum$ Saturated	29.25 ± 0.37^{b}	28.62 ± 0.36^{b}	26.38 ± 0.41^{b}	0.000
$\sum$ Monounsaturated	54.80 ± 0.44^{b}	55.90 ± 0.42^{b}	59.12 ± 0.48^{a}	0.000
$\sum$ Polyunsaturated	15.95 ± 0.39^{a}	15.49 ± 0.38^{ab}	14.50 ± 0.43^{b}	0.054

a,b,c Means with different superscript differ significantly.

The fatty acid profile was significantly affected by growth in the AL pigs fed acorns and grass. From the first sample at 90 kg through the slaughter weight at 110 kg BW, all fatty acid contents were significantly affected with the exception of margaric (C17:0), C18:2, arachidic (C20:0), and eicosamonoenoic (C20:1) acids. In general, the proportion of saturated fatty acids decreased 2.9%, the proportion of monounsaturated fatty acids increased 4.3% and the proportion of polyunsaturated fatty acids decreased 1.5%.

The most noticeable features were the evolution of C16:0, C18:0, C18:1, and C18:2, the fatty acids whose proportions are used as criteria for the evaluation of the raw matter of AL pig from “montanheira”. Considering the body mass gain from the beginning to the end of the experiment (20 kg BW): (i) the proportion of C16:0 decreased 2.1%, to 17.36% (reference limit < 21.3%); (ii) the proportion of C18:0 decreased 0.7%, to 7,25% (reference limit < 9.8%); (iii) the proportion of C18:1 increased 4.9%, to 53.53% (reference limit > 54%); and (iv) the proportion of C18:2 decreased 1.2%, to 12.93% (reference limit < 9.8%). Chemically, acorn is very rich in carbohydrates and has an amount of ~7% crude fat, with a composition in C18:1 of about 63% (Almeida *et al*. 1993). So, the increase of C18:1 in fat depots is explained by the ingestion of acorn and the deposition of dietary C18:1. This deposition had a dilution effect on the other fatty acids. In AL pigs bred in traditional grazing conditions (acorn + grass), Neves (1998) reported similar results: between 90 and 130 kg BW, an increase in the proportion of C18:1 (from 46.3 to 53.7%), and the corresponding reduction in the proportion of C16:0 (from 21.4 to 19.2%), C18:0 (from 9.8 to 8.5%), and C18:2 (from 13.9% to 11.5%).

In farm conditions animals must walk during all day looking for acorns. The intensity of the exercise made by these animals depends on the population of trees, the quantity of acorns by tree, and the geography of the region (mountains and hills). During grazing, it could happen that the most important fatty acid (C18:1) could be metabolized to produce energy rather than used to fat synthesis. The oxidation rates of lauric (C12:0), myristic (C14:0), C16:0, C18:0, C18:1, C18:2, C18:3, and C20:4 were studied in weanling rats by Leyton *et al*. (1987). In this animal model, C12:0 was the most efficient energy substrate among the saturated fatty acids: the longer the chain length of the saturated fatty acids, the slower the rate of oxidation. On the other hand, C18:1 was oxidized at a remarkably fast rate, similar to that of C12:0. Of the n-6 essential fatty acids studied, C18:2 (n-6) was oxidized at a faster rate than any of its metabolites, with C20:4 (n-6) being oxidized at the slowest rate. Finally the rate of oxidation of C18:3 (n-3) was almost as fast as that of C12:0 and C18:1. In the experimental conditions of this work, pigs were fed acorns distributed by hand. Therefore, the only exercise made by these animals was due to behaviour characteristics and grazing, and limited to the area of 2 ha. So, we may assume that the energy consumption for exercise was limited and therefore there was no need of selective fatty acid oxidation to produce energy.

CONCLUSIONS

With an accretion of only 20 kg BW in AL pigs fed grass and 4 kg of acorn daily, it was possible to obtain a proportion of C18:1 in dorsal subcutaneous adipose tissue similar to the one demanded by the criteria for the classification of these pigs as pigs from “montanheira”. It was also registered a proportion of saturated fatty acids within the limits demanded by the mentioned criteria. However, the amount of C18:2 remained ~3% above the reference limit. This could be explained by the fact that when pigs don’t make intense exercise they don’t metabolize the C18:1 for energy production and are able to use it for adipose tissue synthesis.

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ALPHA-TOCOPHEROL CONTENT ON THE SEMIMEMBRANOSUS MUSCLE OF ALENTEJANO PIGS REARED IN INTENSIVE AND EXTENSIVE CONDITIONS

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SUMMARY - This trial was undertaken to determine the effect of the rearing system (intensive vs. extensive) over the content of antioxidants (alpha-tocopherol) in the meat of Alentejano pigs. Sixteen Alentejano pigs with an average body weight (BW) of 60 kg were divided in two groups: Group **I** (n=10) was allocated in open-air individual pens, while Group **II** (n=6) was maintained in a 3 ha natural pasture. Both groups were fed a commercial diet (3100 kcal ED, 14% CP, and 20 mg/kg alpha-tocopherol) at 85% *ad libitum*. All pigs were killed at 100 kg BW and carcasses were refrigerated (24 h at 4ºC). Samples of semimembranosus muscle were obtained, stored under vacuum, and frozen (-30ºC) until analysis. The content in alpha-tocopherol was estimated by HPLC. Results show a significant effect (P<0.001) on the semimembranosus content of alpha-tocopherol. Group **II**, with access to pasture, presented content in alpha-tocopherol of 2,81 ppm, about 2,4-times higher than Group **I**, without access to pasture (1,18 ppm). Such results suggest a beneficial effect of the extensive rearing system over the oxidative stability of the meat of Alentejano pigs, with repercussion on the technological quality of the meat and may be useful to discriminate the finishing feeding system.

Key Words: Alentejano pig, alpha-tocopherol, Semimembranosus, rearing system

INTRODUCTION

The Alentejano (AL) pig production is characterized by the fact that the animals are reared outdoors during almost the time of the production cycle. Nowadays, the production of Alentejano pigs fulfills a double function: it provides meat for the manufacture of cured products and for fresh consumption. The manufacture industry requires pigs reared in traditional extensive system “montanheira”, slaughtered at 140-160 kg BW, with 18-24 months of age. The emerging market of the fresh meat DOP requires animals with 90-120 kg LW, obtained at 10-12 months of age and implies that animals must be reared extensively. Depending on the period of the year/production cycle the ingestion of grass depends on its availability and it is controlled by the animals. The consumption of grass could vary between 1 kg to 1,5 kg.

Tocopherols (beta-, gamma- and alpha-tocopherol) have been found in plants, but alpha-tocopherol seems to be the most abundant and biologically active species in chloroplast membranes (García-Plazaola and Becerril, 1999). Tocopherols are a group of potent lipid-soluble antioxidants and the feeding background affect the deposition of tocopherols (mainly the alpha form) (Rey et al., 2006**).** The intake of grass and acorns by free-range reared pigs has recently been associated with the incorporation of plant phenolics in the animal tissues which could enhance their oxidative stability **(**Cava et al., 1998). Quantification of the tocopherols content as affected by either the time of free-range feeding or the weight gained during the fattening period outdoors could be used as a discriminant for different pig meat qualities (Rey et al., 2006).

This trial was undertaken to determine the effect of the rearing system (intensive vs. extensive) over the content of antioxidants (alpha-tocopherol) in the meat of Alentejano pigs.

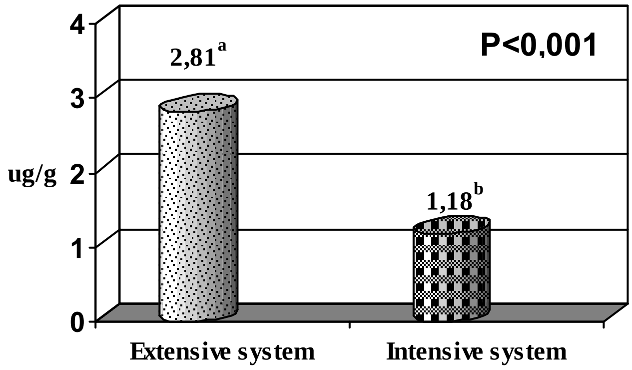
MATERIAL AND METHODS

Sixteen Alentejano pigs with an average body weight (BW) of 60 kg were divided in two groups: Group **I** (n=10) was allocated in open-air individual pens, while Group **II** (n=6) was maintained in a 3 ha natural pasture. Both groups were fed a commercial diet (3100 kcal ED, 14% CP, and 20 mg/kg alpha-tocopherol) at 85% *ad libitum*. All pigs were killed at 100 kg BW and carcasses were refrigerated (24 h at 4ºC). Samples of semimembranosus muscle were obtained, stored under vacuum, and frozen (-30ºC) until analysis. The content in alpha-tocopherol was estimated by HPLC.

RESULTS AND DISCUSSION

Figure 1. shows the amount of alpha-tocopherol in semimembranosus muscle from pigs reared in natural pasture and in individual pens.

Figure 1. Alpha-tocopherol concentration in semimembranosus muscle.



Pigs reared in extensive system presented an higher amount of alpha-tocopherol 2,81 ppm vs. 1,18 ppm in intensive system, about 2,4-times higher**.** Rey et al. (2006) reported that tocopherol accumulation differed with the type of feeding (commercial food v. acorns/acorns and grass). However, Daza et al. (2005) confirmed that muscles from Iberian pigs subjected to dietary supplementation with 200mg/kg alpha-tocopherol contain similar levels of such antioxidant than those from free-range reared Iberian pigs. If it is possible to obtain the same concentration with dietetic supplements to improve the quality characteristics of Alentejano meat and products, according Ventanas et al., (2007) pigs reared in confinement by using enhanced mixed diets was partially fulfilled. On one hand, the dietary supplementation with tocopherol in oleic acid-enriched diets is a successful strategy to increase the levels of alpha-tocopherol and oleic acid in Iberian-dry-cured hams. On the other hand, the hams from pigs fed on this enhanced diet do not attain the intramuscular fat levels of hams from Iberian pigs fed on acorns which could be considered a serious drawback since this parameter infuences essential sensory features of Iberian hams such as the fat oiliness the juiciness and the flavour.

CONCLUSIONS

Such results suggest a beneficial effect of the extensive rearing system over the oxidative stability of the meat of Alentejano pigs, with repercussion on the technological quality of the meat and may be useful to discriminate the finishing feeding system since the amount of alpha-tocopherol was 2,4 times higher in pigs reared in extensive system.

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EFFECT OF PASTURE ON MEAT AND FAT QUALITY IN CINTA SENESE PIGS

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SUMARY - Cinta Senese (CS) is currently bred with different rearing and feeding techniques that consequently determine variability in product characteristics. The aim of our paper was to test the effect of grass pasture on tissue composition of sample joint and on physical-chemical traits of meat and subcutaneous fat. Sixteen finishing CS pigs were divided into two groups: 8 animals (P) were reared on pasture with an integration of 1.4 kg/d per head of mixture usually employed by the farmer; 8 animals (C) were reared in confined area and fed exclusively the same mixture (2.6 kg/d/head). Both groups showed appreciable meat tenderness with no significant differences (shear force of 7.26 *vs* 6.69 kg on raw meat and 7.55 *vs* 8.05 kg on cooked meat, for P and C respectively). High values of intramuscular fat in *Longissimus lumborum* (4.1% *vs* 4.9%) and *Psoas major* (2.6% *vs* 3.0%), for P and C respectively, were recorded. Subcutaneous fat of P revealed higher percentages of C18:3 (0.8% *vs* 0.6%, P=0,004), even if PUFA did not exceed 15%. Results underlined the appreciable characteristics of CS meat fed on pasture and the feasibility of reducing the use of cereals.

Key words: Cinta Senese pig, grass pasture, meat quality, fatty acids.

INTRODUCTION

Cinta Senese (CS) pigs are generally reared under extensive conditions and with different feeding techniques. Pasture plays a fundamental role in pig outdoor husbandry and could extend quality properties with sustainability attributes. The aim of this paper was to test the effect of grass pasture on tissue composition of sample joint and on physical-chemical traits of meat and subcutaneous fat.

MATERIAL AND METHODS

The study was carried out in an organic farm near Pisa. Fattening of Cinta Senese pig happened in large areas where rest and feeding spaces were provided. At start 16 animals were divided in two groups (each with 3 castrates and 5 females) homogeneous for body weight: “concentrate group” - “C” - fed concentrate usually utilised in the farm (2.6 kg/pig/d) and “pasture group” - “P” - fed on grass pasture (6.2-7.3 q/ha respectively at the beginning and at the end of the trial) with an integration based on the same concentrate (1.4 kg/pig/d). Concentrate consisted in barley (40%), corn (20%), *Vicia faba minor* (15%), wheat bran (15%) and wheat bran shorts (10%). Pasture sampling (Numata, 1982) resulted in lucerne, oat, lolium and clover. Chemical composition of feedstuffs is reported in Table 1.

Body weight was periodically recorded and slaughter occurred between 36 and 160 days from the beginning of the trial: this wide range was planned to test the effect of the duration of grass pasture on meat and carcass quality. Each slaughtering regards the same number of animals belonging to the two diets. At slaughter, live and carcass weights were recorded. *In vivo* performance and carcass traits are reported in previous paper (Giuliotti *et al.,* 2007).

Sample joint (loin from 2nd to 5th lumbar vertebra, comprehensive of surrounding backfat) was taken from the right side and dissected in the major tissues. Physical and chemical properties (chemical composition, pH, water holding capacity, Warner Bratzler shear force, colour; ASPA, 1996;

Grau and Hamm, 1952; Boccard *et al.*, 1981) were determined on Longissimus Lomborum (LL) and Psoas Major (PM); colorimetric coordinates (L*, a*, b*) were measured also on the backfat. Total lipids (Folch *et al.*, 1957) and fatty acid composition (Morrison and Smith, 1964) on outer layer of backfat were also determined.

Data were analysed by ANCOVA (SAS, 2003) including in the model diet, sex and trial day, by estimating the LS-means at the average day of trial.

Table 1. Chemical composition of feeds.

		Mixture	Grass
Dry Matter	%	88.5	23
Crude Protein	%	12.7	3.54
Ether Extract	%	2.7	0.65
Crude Fiber	%	4.47	4.42
Ash	%	3.96	2.59

RESULTS AND DISCUSSION

Sample joint composition (table 2) did not show any significant differences between the two groups. Generally, lean to bone ratio was higher than the value found in the Cinta Senese by Franci *et al.* (2005).

As physical trait is concerned (table 3), no differences between groups were found and, generally, meat showed appreciable tenderness. Probably, this result is due to the high values of intramuscular fat in *L. lumborum* (4.1% in P *vs.* 4.9% in C) and *Psoas major* (2.6% in P *vs.* 3.0% in C).

For acidic composition, significant differences were detected only for C18:3, PUFA n3 and n6/n3; PUFA did not ever exceed the threshold of 15% (table 4), above which fat quality can be negatively affected.

CONCLUSIONS

Finishing Cinta Senese pigs on grass pasture produced high quality products with an appreciable polyunsaturated fatty acids content. The results can suggest the substitution of almost the half part of the concentrate with grass in the diet even in finishing period, enhancing the role of an appropriate resources management in extensive rearing.

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Table 2. Sample joint composition.

		Control	Pasture	S.E.
Sample joint	g	2895	2845	130.75
Subcutaneous fat	%	39.00	39.10	1.84
-Outer layer	%	15.01	16.24	0.46
-Inner layer	%	23.99	22.86	1.75
Intermuscular fat	%	5.93	4.38	0.90
Muscle Psoas major	%	9.80	9.83	0.46
Muscle *Longissimus lumborum*	%	28.62	28.71	0.87
Other lean	%	7.16	7.52	0.73
Bone	%	9.49	10.44	0.44
Area *Longissimus lumborum*	cm^2	391	381	25.70
Lean/Bone		4.84	4.47	0.23

Table 3. Physical traits of L. lumborum.

		Control	Pasture	S.E.
Drip loss	%	4.78	4.79	0.674
Cooking loss	%	25.89	25.6	1.931
Free water	cm^2	104.87	86.06	10.411
Shear force on raw meat	kg	6.69	7.26	1.052
Shear force on cooked meat	kg	8.05	7.55	0.946
Color: - L*		48.56	49	1.099
- a*		13.85	13.48	0.673
- b*		4.45	4.47	0.464

Table 4. Main fatty acid composition (% of total) of outer layer backfat

	Control	Pasture	S.E.
C16	23.8	23.86	0.324
C18	11.34	12.3	0.419
C18:1	48.34	47.58	0.434
C18:2	10.66	10.58	0.324
C18:3	0.56 a	0.78 b	0.049
C20	0.09	0.06	0.014
C20:1	0.9	0.9	0.026
C20:2	0.39	0.35	0.028
C20:3	0.07	0.06	0.013
C20:4	0.05	0.05	0.008
SFA	36.71	37.62	0.718
PUFA	11.74	11.82	0.376
PUFA N6	11.18	11.04	0.352
PUFA N3	0.56 a	0.78 b	0.049
PUFA/SFA	0.32	0.32	0.016
n6/n3	21.55 a	14.79 b	1.235

a, b = $P<0.05$

THE "CINTA SENESE" BREEDING SYSTEM: PRODUCTION SYSTEM, BREEDING STRATEGIES AND PERSPECTIVES OF VALORIZATION

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SUMMARY As one of the dynamic livestock segments, the Cinta Senese pig breeding is increasingly recognised as an opportunity to enhance competitiveness and promote sustainable production and land use practices. These patterns have prompted ARSIA (*the Regional Agency for supporting rural development and innovation*) to try to explore in depth the Cinta Senese breeding system. The field analysis has involved a sample of 35 farms. Research activities thus far has focused primarily on the farms structure and organisation systems. Specific attention has been paid to better understand extensive cattle system, focusing on the extreme heterogeneity of forest management ways. Furthermore, a little sharing behaviour has emerged from the feed ratio management, the cattle weight of slaughtering and the marketing and promotion strategies.

Key words: Cinta Senese pig, extensive breeding, local/authchonous breed, marketing strategies.

INTRODUCTION

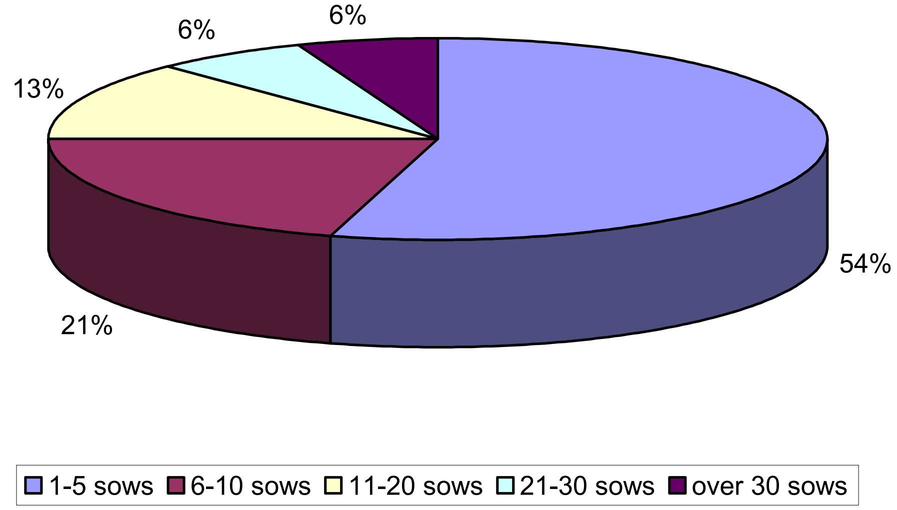
ARSIA (Regional agency for agricultural development and innovation in Tuscany) has realized a survey that has concerned 35 Cinta Senese pig breedings with the aim of understanding and improving this old breed recovery. The study wanted to notice technical information on breeding management, productive performances, marketing strategies and to point out breeders' technical and commercial needs.

According to breeders associations data (APA), in 2006 there were in Tuscany 155 Cinta Senese farms with nearly 1500 sows 250 boars. Considering that in 1998 the total ammount of these pigs was very low (about 120 sows and 20 boars) the growth rate of these breedings has been really amazing. In table 1 it is reported the Cinta Senese heritage of the region: most of the breedings are located in the province of Siena (the area gave origin to the race) and in the provinces of Firenze, Livorno Arezzo and Grosseto; the majority of the breedings are very small in term of number of sows, 75% of them have less than 10 sows (Fig. 1).

Table 1. Number of Cinta Senese breedings, and number of sows and boars per each province of Tuscany (APA 2006).

TOTAL	**156**	**1487**	**252**
Province	**Pig breedings**	**sows**	**boars**
Arezzo	23	84	22
Firenze	12	250	30
Grosseto	13	75	16
Livorno	11	244	47
Lucca	4	56	7
Massa Carrara	4	20	4
Pisa	8	40	9
Pistoia	3	17	7
Prato	2	7	2
Siena	76	694	108

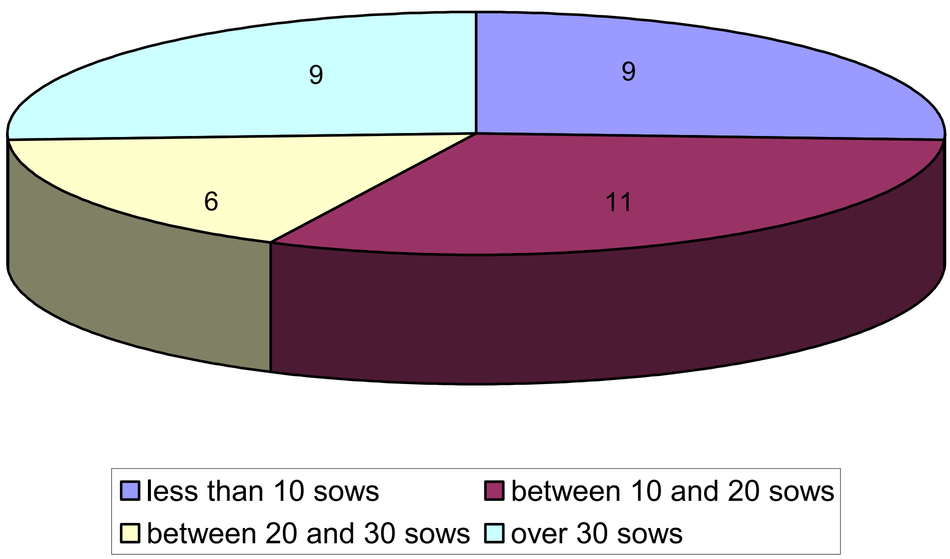
Figure 1. Tuscany: percentage of breedings per number of sows classes.



Farm sample

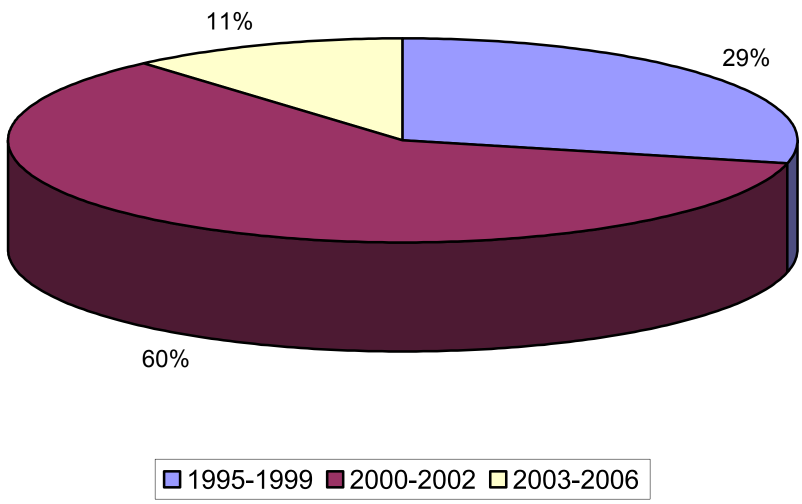
The sample of farms has been chosen in considering the number of the sows and their location, preferring the biggest breedings situated in the mid-south of Toscany. The size of breedings is shown in figure 2 where the sample is divided into groups in relation to the number of the sows.

Figure 2. Farm sample: number of pig breedings divided into classes of sows number.



The majority of pig-farms concerned by the survey do the breeding and rearing phase and also the fattening phase; only two of them are specialized in producing piglets; all of them are extensive outdoor breedings. As shown in figure 3, in many cases the beginning of the activity is quite recent with an explosion of breedings between 2000 and 2002. The 35% of Cinta Senese farms was born as a new activity while in the other cases breeding represent a new sector of a pre-existing farm.

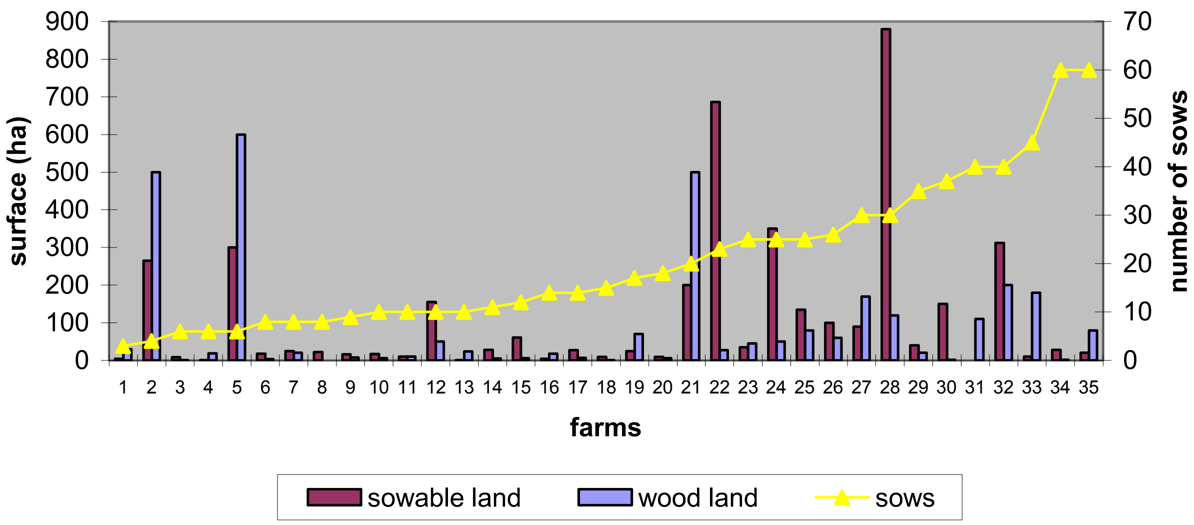
Figure 3. Beginning of the activity for the Cinta Senese farms of the sample.



Breeders are young, in comparison with the average of Italian farmers (30% of them have less than 40 years old); they have a good instruction level (15% of them have a degree and 65% a high school certificate) and they are represented by women in the 20% of the sample.

Dimension and land use of the farms are varied; as illustrated in figure 4, total surface, cultivable surface, wood surface and the number of sows are associated in many combinations. There are big farms in which most of the land is covered by wood and the number of sows is low, there are big farms where the majority of the surface is sowable land and the number of sows is intermediate and farms in which all the the land is covered by the wood and the number of the sows is high. As for the wood it is interesting to notice that there are 7 farms (20% of the sample) where its surface is nearly absent.

Fig. 4. Sowable land, wood land and number of sows

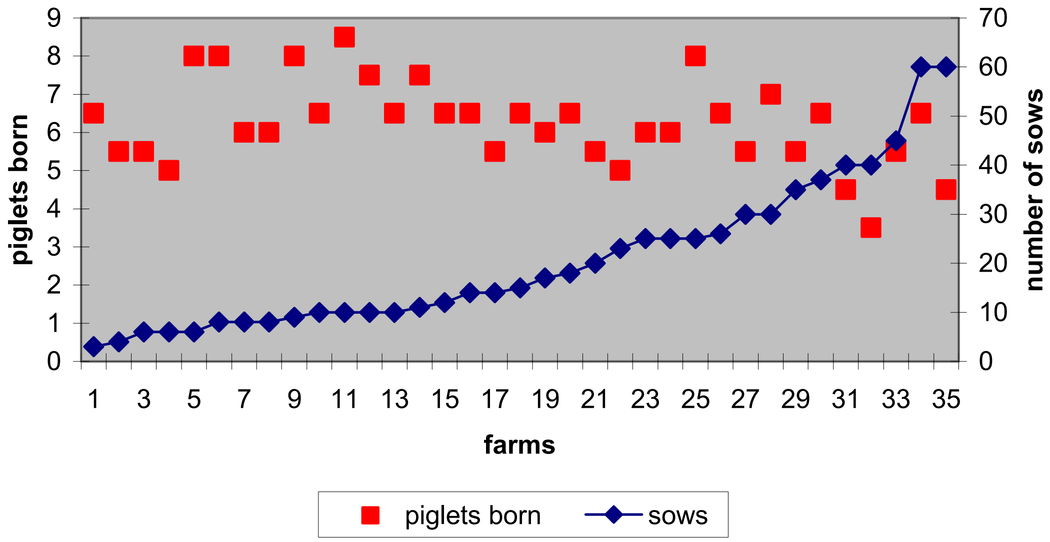


Reproduction

When all the breeders have to choose a boar, they verify the consanguinity with ANAS web site service, taking into great consideration the problem of genetic variability lack. In branding piglets (registering genealogy and verifying breed's phenotypical characteristics) there are no problems of identification because usually sows litter in individual fences, where they stay until piglets are weaned. Only in four breedings the delivery takes place in big fences where more sows are put together, while other four farms practice indoor delivery.

Mating period is planned by fifty percent of the breeders to concentrate births during spring and autumn, while in other cases sows and boars are never separated. The average of piglets born per sow is about six, but incresing the dimension of the breeding this value tends to decrease, as shown in figure 5.

Fig. 5. Number of piglets born in relation with number of sows of the pig-farm



Piglets mortality depends on pneumonia for 26% of the breeders, on crushing by sows for 14% of the breeders and on predators (foxes and wolves) for 12% of the breeders. Others causes of death can be high temperature in summer time and the scarcity of sow milk.

Forest grazing

Traditional Cinta Senese breeding is an outdoor rearing with pasture on woods of chestnut and acorn. The use of this natural resource have many implications that concern the environment (taking into consideration forest grazing charge) meat quality (using chestnut and acorn as fodder) and marketing (consumers associate this old breed to extensive rearing in the wood).

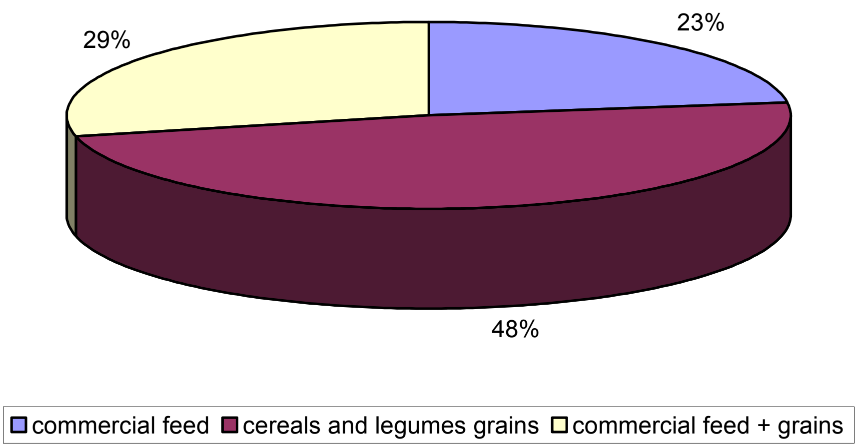
In the farms studied, the forest is used in different manner by breeeders: 60% of them keeps pigs grazing in the forest all over the year, 20% sends the pigs to forest mostly in the finishing phase and when chestnuts and acorn are available; as said previously the rest of the farm sample (about 20%) doesn't have wood and uses only herbaceous pastures.

Forest is used with a turnover system in 55% of the breedings, where the wood is divided into sector with electric fencing, while in the other cases all the surface is permanently grazed. These practices have big impact in forest renovation capacity and in soil erosion, themes considered of primary importance by the 50% of the farmers. Finally the wood is an important source of fodder in the 45% of the breedings.

Nutrition

Pig ration, as reported in figure 6 is composed of commercial feeds and/or cereals and legumes grains: 23% of the breedings uses only commercial feeds while 50% uses grains of cereals (wheat, oat, barely, corn) and legumes (pea and especially broadbean). Grains are sometimes milled or soaked in water but more frequently they are given to pigs without any treatment and this causes a big loss of the ration nutritional value.

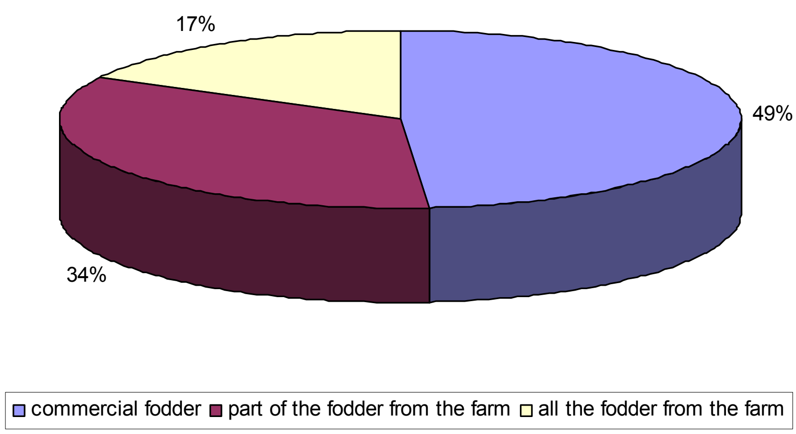
Fig. 6. Percentage of breedings in relation with pig ration.



Commercial feeds used are specific for pig and sometimes for Cinta Senese and their formulation is processed in relation with pig development phase. Feeds contain several ingredients: cereals (wheat, oat, barely, corn), wheat bran, legume grains (soybean, broadbean, pea), salts (calcium phosphate, calcium carbonate, sodium chloride) and sometimes amino acids (lysine, methionine).

Concerning feed origin, figure 7 shows that only 15% of the breedings are self-sufficient while in most of the cases ration ingredients are not produced by the farm.

Fig. 7. Percentage of breedings related to fodder origin

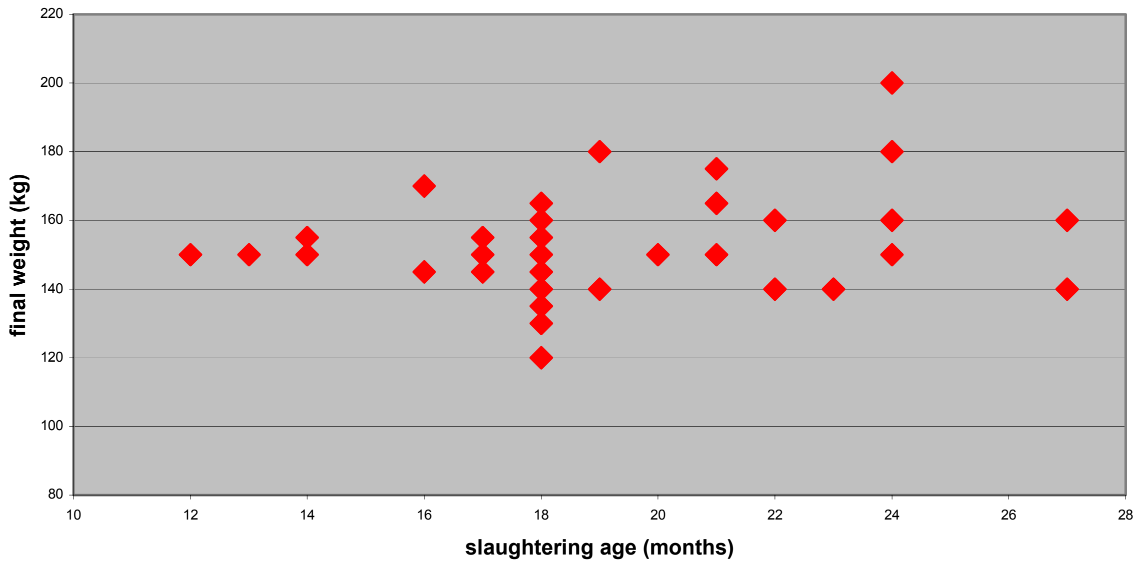


The feed amount given to Cinta Senese pigs varies from two to three percent of body weight but in some period can be lower if there is the possibility to integrate diet with pasture.

In 65% of the breedings there is only one kind of ration given in different quantity in accordance with the pig developing phase. Besides in the 35% of the pig farms the diet varies by increasing proteins in the growing stage.

Slaugthering age and final weight (Figure 8) are quite differentiated because of the different strategies of managing the herd and the different feed ration chosen by farmers. 150 kg of final weight is reached in the majority of the breedinds between 18 and 22 months, but in some cases farmers need more time to fatten their pigs.

Fig. 8. Age of slaughtering and final weight



Products processing and sale

About 65% of the breedings processes their meat, producing tipycal Tuscan salami (ham, bacon, lard..) and also particular products obtained following ancient recipes or following north of Italy traditional way of processing. The majority of these breedings have set up their own workshop to process the meat while the others turn to factories producing salami. Products, addressed to high level of consumers, are sold especially to restaurants and grocer's fashionable shops; only one producer supplies supermarkets and two farms export salami and meat to Japan and north Europe. In general, breeders that process their meat are quite satisfied with the prices they obtain, even if there is still a big variability on the market; for example the price of whole ham can vary from 28 to 50 €/kg from one farm to another.

For the farmers that don't process their products the most important trade channel is represented by factories producing salami: very rarely fatty pigs are sold to other breeders provided with a processing workshop or sold to privates. Prize per kg (the weight of a body alive) is around 2,50 € but it can fall under 2,00 € in periods of big offer. For this reason breeders that don't have the possibility of processing their meat, express their discontent about the prizes, complaining that the market provides less profits than few years ago.

Concerning strategies to enhance the value of Cinta Senese products, breeders hope that DOP "Suino Cinto Toscano", that is going to finish its procedure to be approved by EU, wil be useful to reduce frauds, to level products quality and to peg prices. Organic certification, obtained by 35% of the farms concerned by this survey, doesn't bring big advantage in selling products but allow to comply with Rural Development Plan raising contributions and funds.

Breeders demands

Breeders needs, in term of technical assistance are very different, responding to big differences in farm dimension and way of managing. Common requests this survey has noticed concern:

- processing technique: processors need to rediscover ancient recipes and to reduce the use of preservatives;

- slaughtering: in Toscany there are only few slaughterhouses (high carriage fees) that sometimes don’t respect animal welfare.

Other problems which have come out of interviews, are related to feeding techniques, where it is important to identify the best relation between pig age and final weight, in evaluating meat quality and production cost.

Besides, farmers complain the lack of promotion strategies to let consumers know Cinta Senese and appreciate its particular and not standardized taste.

CONCLUSIONS

This survey represents a picture of Cinta Senese breedings in Tuscany and shows how this division has answered to the crisis of the last few years. In varied situations some farms reacted to prices reduction in investing to direct processing of their meat, while little farms are discussing about the possibility of creating a cooperative structure to process their goods, otherwise they would fail .

Finally it is important to highlight that Cinta Senese represents, in the national context, an important example of recovery and relaunching of an ancient breed that was very close to extinction. The success of the improvement of Cinta Senese depends on breeders’ and processors’ perseverance, on the promotion and contribution done by associations and public institutions, and on the scientific activity of the University of Forence.

IN VITAM AND *POST MORTEM* PERFORMANCES OF "NERO SICILIANO" FATTENING PIGS FED WITH DIFFERENT DIETS

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SUMMARY - The in vitam and post mortem effects of two different diets, Acorn (A) *vs* Barley (B), were examined in 24 "Nero Siciliano" pigs over 90 days during the fattening period. The animals were assigned to two homogeneous groups of 12 animals respectively. The individual baseline weight was recorded and the average daily gain (ADG) was calculated every month. After slaughtering, the thickness of the dorsal fat tissue was measured; yield, pH_1 and pH_U were also determined. A sample cut was taken from the loin and dissected in order to analyze the major tissues. Data were subjected to the GLM-SAS. The results (pH_1: A 6.28 *vs* B 6.12; pHu: A 5.77 vs B 5.63; final life weight: A 109.93 kg *vs* B 120.45 kg; ADG: A 346 g/d *vs* B 465 g/d) indicated a statistically significant ($P \leq 0.05$) influence of the diet during fattening.

Key words: Nero Siciliano pig, barley, acorn, *in vitam* performances, carcass traits.

INTRODUCTION

The "Nero Siciliano" pig is a native breed that has been reared in Sicily for a long time. Recently there has been an enhanced demand for "Nero Siciliano" products, and therefore the number of breeding animals is being increased. According to official registration, the present population comprises about 300 sows, although the actual numbers might differ from the official data. The objective of this study was to examine the *in vitam* and *post mortem* feeding effects in "Nero Siciliano" fattening pigs fed on different diets.

MATERIAL AND METHODS

The trial was carried out with 24 "Nero Siciliano" pigs, bred in the Nebrodi mountain region of Sicily. Animals were assigned to two different groups called Acorn (A) and Barley (B), each consisting of 12 animals, homogenous for sex (castrated males) and body weight (BW 79.48 ± 0.15 kg). Animals of group A were kept in a wooded area of 12 hectares, appropriately enclosed, and fed with acorn during the fattening period (90 days). Animals of group B were reared within an open-air system in the same rural region and fed with germinated barley on a basis of 2.5 kg/pig/d during the fattening period (90 days). At the beginning of the trial, individual microchips (Portorider) were implanted subcutaneously underneath the ear of each animal in order to facilitate an electronic identification from a distance. The baseline weight of each pig was recorded and the weight was measured every month using an electronic weighting machine (Laumas Elettronica) to determine the average daily gain (ADG). After 90 days of fattening and a fasting period of 18 hours (ASPA, 1991) the animals were slaughtered. The carcasses were cut lengthwise into two halves. The yield was calculated for every carcass and the thickness of the dorsal fat tissue was measured at the first (1T) and the last (UT) thoracic vertebra, as well as on the upper *Gluteus medius* muscle (GM). pH_1-values (measured 45 minutes after slaughtering) and pH_U-values (measured 24 hours after slaughtering) from the muscle *Longissimus lumborum* (LL) were determined by a pH-meter (WTN 597-S) equipped with penetration probe. A sample cut was taken from loin (including lumbar vertebrae 2^{nd} – 5^{th}) and dissected in order to analyze the major tissues. The obtained data were subjected to the GLM procedure of SAS (2001).

RESULTS AND DISCUSSION

The *in vitam* parameters were significantly influenced by the feeding method used during fattening. In fact, in the course of the trial (Table 1) the measured ADG were 346 g/d for animals in the A group and 465 g/d for animals in the B group (P = 0.004).

Table 1. *In vitam* and *post mortem* traits of Nero Siciliano pigs fed with either Barley or Acorn during the fattening period

	Acorn (A)	Barley (B)	*P*
ADG (g)	346	465	*
Slaughtering body weight (Kg)	109.93	120.45	**
Yield (%)	80.45	80.96	NS
pH_1(45')	6.28	6.12	**
pH_U (24h)	5.77	5.63	**

NS: not significant; * P ≤ 0.05; ** P ≤ 0.01.

As shown in Figure 1, the two groups, starting from identical baseline weights (79.48 kg), showed a noticeably different weight gain during the first month (day 30: A 92.04 kg *vs* B 101.77 kg), with re-approaching weights around the 60^{th} (day 60: A 108.69 kg *vs* B 112.59 kg) and arrived at slaughtering with significantly different average weights (A 109.93 kg *vs* B 120.45 kg; P = 0.004).

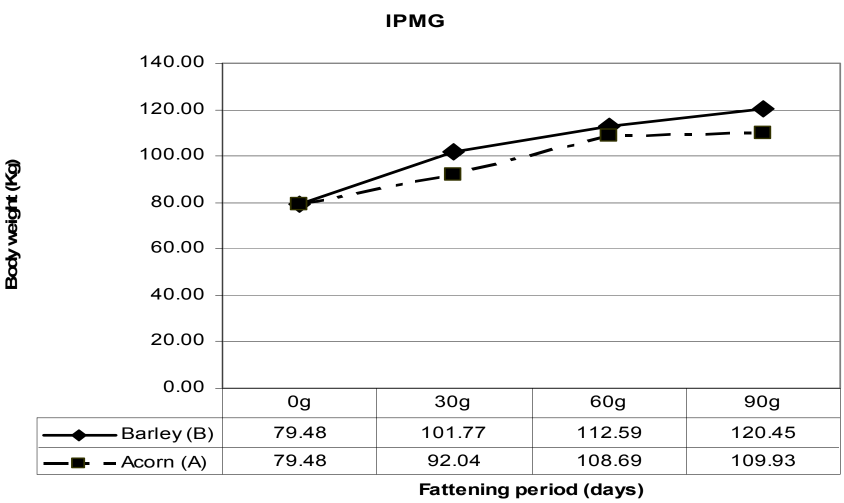

Fig.1. Relationships between fattening duration and body weight

Most likely, these results are due to the different diets used during the trial. The yield, as shown in Table 1, exceeded all expectations in both groups (A 80.45% *vs* B 80.96%) and was higher than the one found by D'Alessandro *et al.* (2007) for "Nero Siciliano" pigs fed with barleycorn and citrus pulp (72.9%). The yield also exceeded the one found by Campodoni *et al*. (1999) for Large White x Cinta

Senese cross breeds raised outdoors in a forest region on pasture with partial food integration (79.89%). As to the pH-values measured at 45 minutes after slaughtering, risk indicator for the possible presence of PSE-(Pale/Soft/Exudative)-meat, Table 1 shows highly significant results (A 6.28 *vs* B 6.12; p = 0.005), comparable with the ones found by Zumbo *et al.* (2002) for pigs of the same genetic type (6.16) reared under extensive conditions, and lower than the ones found by Pugliese *et al.* (1999) for Large White x Cinta Senese cross breeds (6.39). Also, the pH-values measured 24 hours after slaughtering, used to exclude the presence of DFD-(Dark/Firm/Dry)-meat, were shown to be significant (Table 1) and higher than the ones found by the authors mentioned above (Zumbo *et al*., 2002: 5.50; Pugliese *et al*., 1999: 5.57). As illustrated in Table 2, measurement of the dorsal fat showed a lower thickness at every measuring site for animals that had been fed on acorn; however, only measurements at the last thoracic vertebra turned out to be statistically significant (UT: A 2.8 cm *vs* B 3.7 cm; P = 0.02).

Table 2. Dorsal fat thickness measured after slaughtering of Nero Siciliano pigs fed with either Barley or Acorn during the fattening period. (Mean value ± SD)

	Acorn (A)	Barley (B)	*P*
Dorsal fat at the first (1T) thoracic vertebra (cm)	4.05±1.54	4.9±0.86	NS
Dorsal fat at the last (UT) thoracic vertebra (cm)	2.8±0.95	3.71±1.04	*
Dorsal fat at the upper gluteus medius muscle (GM) (cm)	3.23±1.07	3.9±0.86	NS

NS: not significant; * P ≤ 0.05.

The dissection of the sample cut (Table 3) also revealed the positive effects of the acorn diet used during the finish phase since A group carcasses showed higher percentages of lean cuts at the expense of fat and bone. This fact might be particularly influenced by the diet (A 18.58% *vs* B 21.12%; p = 0.01).

Table 3. Tissue composition of the sample cut of Nero Siciliano pigs fed with either Barley or Acorn during the fattening period. (Mean value ± SD)

	Acorn	Barley	*P*
Sample cut weight (g)	967.40±190.35	996.91±301.38	NS
Lean (%)	69.8±3.90	67.74±3.03	NS
Fat (%)	11.6±3.46	9.98±2.23	NS
Bone (%)	18.58±2.35	21.1±1.74	*

NS: not significant; * P ≤ 0.05.

Even though factors like the complexity of the breeding environment, the variety and variability of climatic conditions and feed provision in the mountain region of the Nebrodi should not be underestimated, the overall results are an incentive for using feedings that can be found on the spot; furthermore, they are useful in pursuing a twofold objective: to obtain high quality products from the "Nero Siciliano" pigs and to protect the forest, thus reinforcing its status as an important resource.

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VITAMIN E ADMINISTRATION IN NERO SICILIANO PIGS: EFFET ON THE OXIDATIVE STABILITY OF “NEBRODI” CURED SAUSAGE

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SUMMARY - The aim of this study was to evaluate the antioxidant effect of Vitamin E on the stability of “Nebrodi” cured sausages. Sausages were prepared using meat of 18 Nero Siciliano pigs living in plein-air system in the Nebrodi area, divided into two groups called CTR and Vit.E. Every 15 days, the pigs of Vit.E group were treated with d,l-alfa tocopheryl acetate i.m. (200 I.U./head). After slaughter (age=250 days), on the cured sausages, prepared using the standard technology of the “Consorzio di tutela del suino Nero dei Nebrodi”, fatty acid composition at 30 days of seasoning and oxidative stability (TBARs) at 0, 15 and 30 days of seasoning, were determined. SFAs, MUFAs, n6-PUFAs and n3-PUFAs, showed no significant differences ($P>0.05$) as well as Atherogenic (Vit.E=0.5 vs. CTR=0.5) and Thrombogenic (Vit.E=1.39 vs. CTR=1.34) indices. Oxidative stability was unaffected ($P>0.05$) by treatment, even if TBARs trend of Vit.E group showed a slightly better stability than that of CTR group.

Key Words: Nero Siciliano pig, vitamin E, cured sausage, oxidative stability

INTRODUCTION

Lipid oxidation is one of the major causes of quality deterioration of meat, with adverse effect on flavour, colour, texture and nutritional value (Byrne, 2000). In particular, the high levels of intramuscular fat and oleic acid in the meat of pigs belonging to autochthonous race, such as Iberian pigs, are responsible for certain texture and appearance traits which influence consumer’s acceptability (Ventanas *et al.*, 2007). Efficient protection of meat products against oxidative deterioration depends on an optimisation of the process parameters identified at critical points along the production chain such as the use of an antioxidant as a way of reducing the susceptibility of the muscle lipid and myoglobin to oxidation (Daza *et al.,* 2005). Considering the high proportion of unsaturated fats of the Nero Siciliano meat (Pugliese *et al.*, 2004) and consequently its particular susceptibility to oxidative rancidity (Liotta *et al.*, 2007), the aim of this study was to evaluate the effect of Vitamin E on the stability of the “Nebrodi” cured sausages prepared using meat of Nero Siciliano pigs.

MATERIAL AND METHODS

The research was carried out on 18 Nero Siciliano pigs living in the plein-air system in the Nebrodi area. The animals were divided into two groups of 9 (5 males and 4 females) homogeneous for B.W. (35 ± 2kg) and age (150 ± 15 days) called CTR and Vit.E. Animals fed on spontaneous fruits of the undergrowth integrated (3% of B.W.) with concentrate (Dry Matter: 87.5%, Crude Protein: 17.5%, Ether Extract: 3.0%, Crude Fibre: 5.5%, as fed) analysed using the official methods (AOAC, 2000). Every 15 days, the pigs of the Vit.E group were treated (200 I.U./head) with an intra-muscular administration of d,l- alfa tocopheryl acetate (Vitalene E®, FATRO SrL, BO, Italy). After slaughter (age=250 days), on the cured sausages, 30 days of seasoning and 290g of weight (on average), prepared using the standard technology of the “Consorzio di tutela del suino Nero dei Nebrodi”, the fatty acid composition (Chiofalo *et al.*, 2005) was determined; each fatty acid was expressed as percentage of the total identified fatty acids. On the basis of the fatty acid profile, the Atherogenic and Thrombogenic indices of sausages were calculated using the equations proposed by Ulbricht and Southgate (1991). The oxidative stability as measured by formation in Thiobarbituric Acid Reactive Substances (TBARs) was determined at 0, 15 and 30 days of seasoning and measured as absorbance (A) at 532 nm (Faustman *et al.*, 1992). The results were subjected to ANOVA (SAS, 2001), using the following model: $y_i = \mu + a_i + e_i$; where a_i = effect of treatment.

RESULTS AND DISCUSSION

SFAs, MUFAs, n6-PUFAs and n3-PUFAs, showed (Table 1) no significant differences (P>0.05) nor any polyunsaturated fatty acids of nutritional interest of the n6-series, whereas, the essential fatty acid of the n3-series showed a higher value in the Vit. E group than that of the CTR group (Table 2). This could be due to the low levels of vitamin E administered which were not sufficient enough to protect, during the ripening, the high levels of unsaturated fatty acids contained in the sausages (Lòpez-Bote *et al.*, 2005). Consequently, the Atherogenic and Trombogenic indices were not influenced by the d,l- alfa tocopheryl acetate administration (Table 1).

Oxidative stability was unaffected (P>0.05) in a significant way by treatment, even if the TBARs trend of the Vit.E group showed during the seasoning a slightly better stability than that of the CTR group (Figure 1).

The results suggest a further optimisation of dosage and time of vitamin E administration, considering the interest of farmers and technicians for the “Nebrodi” cured sausage.

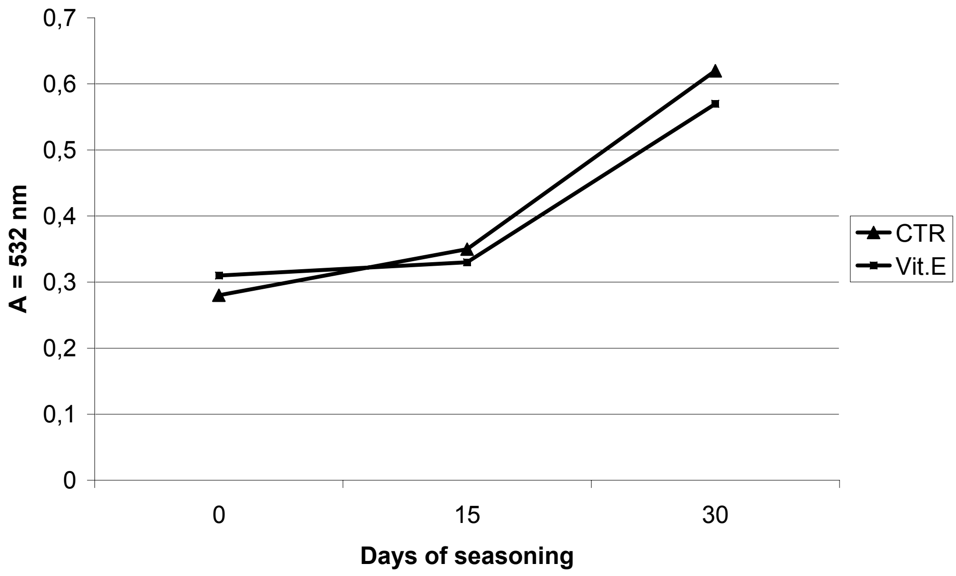
Table 1. Acidic composition and Quality indices of the sausages in relation to the d,l-alfa tocopheryl acetate administration (mean ± SD, Probability).

	Groups		*P*
	CTR	Vit. E	
Saturated (SFA), %	42.39±3.74	43.22±5.08	0.35
Monounsaturated (MUFA), %	44.88±3.96	43.80±4.28	0.33
Polyunsaturated (PUFA), %	12.73±0.44	12.98±1.24	0.33
n3-PUFA, %	0.95±0.11	1.02±0.13	0.19
n6-PUFA, %	11.78±0.39	11.96±1.14	0.36
Unsaturated/Saturated (UFA/SFA)	1.38±0.20	1.31±0.25	0.39
Atherogenic Index (AI)	0.50±0.04	0.51±0.04	0.35
Thrombogenic Index (TI)	1.34±0.22	1.39±0.29	0.37

Table 2. Polyunsaturated fatty acids of nutritional interest in relation to the d,l-alfa tocopheryl acetate administration (mean ± SD, Probability).

	Groups		*P*
	CTR	Vit. E	
Linolenic acid ($C_{18:3n3}$), %	0.67±0.15	0.82±0.07	0.023
Linoleic acid ($C_{18:2n6}$), %	10.96±0.32	11.09±1.05	0.39
Arachidonic acid ($C_{20:4n6}$), %	0.35±0.07	0.37±0.06	0.38

Fig. 1. TBARs trend at 0, 15 and 30 days of seasoning in relation to the diet (mean values)



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DIETARY SUPPLEMENTATION OF ROSEMARY EXTRACT IN GROWING NERO SICILIANO PIGS: EFFECT ON SOME HAEMATOLOGICAL PARAMETERS

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SUMMARY - Natural polyphenols found in the leaves of rosemary (*Rosmarinus officinalis* L.) have potential benefit, because of their potent antioxidant activity and their anticancerogenic and antiviral properties. Active components in rosemary extract are flavonoids, terpenoids, carotenoids. The activities of dietary rosemary extract on some haematological parameters in Nero Siciliano pigs were investigated. 30 Nero Siciliano pigs (16 castrated males and 14 females), during the growing-finishing period (120 days), were divided into two homogeneous groups of 15 each one (8 males and 7 females, LW 42±2 kg), which received (3% of B.W.) the basal diet supplemented with (Rox group) or not (Ctrl group) 1g·kg^{-1} a rosemary extract (ROX P® – Sevecom S.p.A.). On each subject, every 30 d, individual blood samples were taken (K-EDTA venoject®) and some haematological parameters analysed (GENIUS – VET, SEAC ®). Results were subjected to ANOVA. White blood cells count was significantly higher in Rox group (Rox 25.48 x10^9/l vs. Ctrl 22.38 x10^9/l; P=0.021), whereas hemoglobin content was significantly higher in Ctrl group (Rox 13.21 g/dl vs. Ctrl 13.73 g/dl; P=0.048). No significant differences for RBC, Hct, MCV, MCH, MCHC, RDW, Plt, MPV, Pct, PDW values were observed.

Key words: Nero Siciliano pig, rosemary extract, haematological parameters

INTRODUCTION

The prohibited use of antibiotic growth enhancers since the beginning of the year 2006 necessitates to consider alternative substances that may help to support the immune function and health status of farm animals (Bosi and Trevisi 2006) considering that, the maintenance of optimal immune functions, is an important prerequisite for the production of healthy food from animal origin. In this context, recent trends and developments in the area of animal nutrition have been characterized by an increasing interest in the potential impact of plants, herbs and spices on the immune function of animals. In fact, herbs, spices or in general botanicals can have a mesurable impact on the function and reactivity on the immune system (Craig, 1999) even if, the understanding of the specific mode of action and the functional aspects of phytogenic additives still need much more in depth studies. Mechanisms for the interaction of plants with the host's immune system maybe related to intestinal and extraintestinal effects; the different mode of action is related to the variety of active ingredients which cause different reaction of the immune system to ingested plant materials (Smulikowska, 2006). Several herbal product that may enhance the function of the immune system are available (Savoini *et al*., 2003). These includes rosemary (*Rosmarinus officinalis* L.), an herbs rich in flavonoids, with a potent antioxidant activity (Liotta *et al*., 2006; Liotta *et al*., 2007) and anticarcinogenic and antiviral properties (Aruoma *et al*., 1996; Offord *et al*., 1997). In some stressed conditions, dietary rosemary extract might have an effective immunoenhancing action (Babu *et al*., 1998).
The aim of this study was to evaluate the influence of a dietary rosemary extract (*Rosmarinus officinalis* L.) on some haematological parameters in growing Nero Siciliano pigs.

MATERIAL AND METHODS

The research was carried out on 30 Nero Siciliano pigs (16 castrated males and 14 females), clinically healthy and parasite-free, living in "plein air" system during the growing-finishing period (120 days). The animals were divided into two homogeneus groups of 15 each one (8 males and 7 females, LW 42±2 Kg), which received the basal diet (3% of B.W.) as pelletted complete feed (Dry

Matter = 87.5%, on a DM basis: 18.29% Crude Protein, 3.43% Ether Extract, 6.06% Crude Fibre, 7.43% Ash) (A.O.A.C., 2000) supplemented with (Rox group) or not (Ctrl group) 1g kg^{-1} of a rosemary extract (ROX P® – Sevecom S.p.A., Milano, Italy). On each subject, every 30 days, individual blood samples (9mL) were collected from the jugular vein in a vacutainer containing K-EDTA and the following haematological parameters were determined by using an automatic analyser (GENIUS - VET, SEAC®): White Blood Cell Count (WBC), Red Blood Cell Count (RBC), Hemoglobin (Hgb), Hematocrit (Hct), Platelet count (Plt), Mean Corpuscolar Volume (MCV), Mean Corpuscolar Hemoglobin (MCH), Mean Corpuscolar Hemoglobin Concentration (MCHC), Red blood cells Distribution Width (RDW), Mean Platelet Volume (MPV), Plateletcrit (Pct), Platelet Distribution Width (PDW).

The results were subjected to ANOVA (SAS, 2001), using the following model: $y_i = \mu + a_i + e_i$; where a_i = effect of the diet.

RESULTS AND DISCUSSION

Table 1 reports the haematological parameters. No significant differences for RBC, Hct, MCV, MCH, MCHC, RDW, Plt, MPV, PCT, PDW values were observed in relation to the rosemary supplementation. Instead, the mean values of WBC resulted significantly higher in Rox group than in the Ctrl group, and those of Hgb were significantly lower in the animals of the treated group; however, these values are within the physiological ranges also considering the typical breeding system of Nero Siciliano pig.

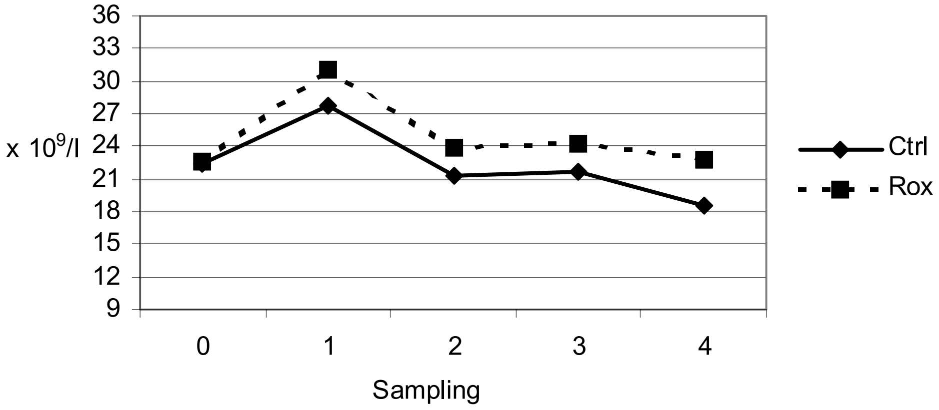
During the trial the trend of the WBC showed no significant differences, even if, in each sampling, the WBC value of the Rox group was slightly higher than that of the Ctrl group (Figure 1).

Table 1. Effect of a dietary rosemary extract on haematological parameters in growing Nero Siciliano pigs (mean±SE)

	Groups		
	Ctrl	Rox	*P-value*
White Blood Cell Count ($x10^9$/l)	22.38±0.90	25.48±0.93	*
Red Blood Cell Count ($x10^{12}$/l)	7.19±0.10	7.00±0.10	NS
Haemoglobin (g/dl)	13.73±0.18	13.21±0.18	*
Hematocrit (l/l)	41.10±0.54	39.91±0.57	NS
Mean Corpuscular Volume (fl)	53.99±1.51	56.97±1.57	NS
Mean Corpuscular Haemoglobin (pg)	19.10±0.17	18.85±0.18	NS
Mean Corpuscular Haemoglobin Concentration (g/dl)	33.38±0.19	33.07±0.20	NS
Red blood cells Distribution Width (%)	17.81±0.17	17.90±0.17	NS
Platelet count ($x10^9$/l)	320.38±19.12	322.39±19.80	NS
Mean Platelet Volume (fl)	9.37±0.09	9.45±0.10	NS
Plateletcrit (%)	0.30±0.02	0.30±0.02	NS
Platelet Distribution Width (%)	15.12±0.19	14.73±0.19	NS

NS: not significant; *P≤0.05

Figure 1. Trend of WBC count in growing Nero Siciliano pigs supplemented with (Rox group) or not (Ctrl group) of a rosemary extract.



The higher significant (P≤0.05) mean values observed for the WBC count in the treated group could be related to the effects of rosemary on immune response (Babu *et al*., 1998). In fact, it was demonstrated that herbs rich in flavonoids, antioxidant and carotenoids, such as rosemary, may enhance immune functions through their ability to act as an anti-inflammatory agent and as an immunestimulant (Craig, 1999).

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PERFORMANCES OF NERO SICILIANO SOWS AND LACTATING PIGLETS

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SUMMARY
The aim of the study was to evaluate some performances of Nero Siciliano sows and lactating piglets. Sows, 7 primiparous and 10 pluriparous, were stabled in single box and fed concentrate *ad libitum*. Before farrowing, on each sow, live weight, thickness of the back-fat, by using ultrasound instruments, number and weight of the piglets were determined. Each piglet was weighed at 5 days of age and, successively, every 15 days until to the weaning (50 day). Data were subjected to ANCOVA considering the variable "parity". Live weight of sows at the farrowing was 139±10 kg, on average, whereas thickness of the back-fat was 4.38±0.32 cm. The number of piglets for each sow was 5.5, with a live weight, at 5 days of age, of 1.5±0.24 kg for females and 1.7±0.25 kg for males and, at the weaning, of 8.6±0.57 kg for females and 8,8±0,77 kg for males, calculating for both an ADG of 137±2.6 g/d for males and 142±2.7 g/d for females, on average. The mean values of the number of teats was 10.5±0.5 for females and 11±0.8 for males.

Key words: sows, piglets, performances, weaning

INTRODUCTION

The knowledge of the sow's performance is the basis of pig farming and the reproductive period is of great interest in order to optimize the phases of feeding (Chiofalo, 1984; Kirwood *et al.,* 1988), gestation (Gadd, 1986) and lactation (King e Dunkin, 1986).
In the context of an integral rural development according to the emerging concept of sustainable agriculture, autochthonous genetic resources have a fundamental and irreplaceable role (Matassino e Grasso, 1996).

The determination of reproductive parameters, known also as vital statistics, is therefore of considerable importance for a knowledge in dept of the productive potentiality of an autochthonous population race, such Nero Siciliano pig, in order to plan the work of selecting and to improve techniques for feeding and reproduction of the this animal (Liotta *et al*., 2006; Liotta, 2006). The aim of the study was to evaluate some performances of Nero Siciliano sows and lactating piglets for the optimization of nutritional management of this period.

MATERIAL AND METHODS

Seventeen Nero Siciliano sows (7 primiparous and 10 pluriparous), registered at the National Pedigree Register, were reared in individual boxes (3x2 metres) for thirty days after birth and, successively, in a plein air system. Sows were fed with the typical feeding system using a mixed feed (Moisture 12%; CP 16.50%, EE 3%, CF 8%, as fed) *ad libitum.* The study lasted 50 days, from the birth to the weaning of piglets.

Considering the close correlation between weight and thickness of the dorsal fat with the reproductive performance, at farrowing on the sows were recorded: live weight using an electronic balance with accuracy ±1 kg; the thickness of back fat found to 6 cm from the midline of the back at the last rib through an ultrasound (Pie-Medical Scanner – 100) with linear probe 6/8 MHZ; total number of piglets born, and those born alive. Subsequently, on the total number of piglets born, was recorded the percentage of the sexes, the number of teats; on each piglet, at 5 days of age until at the weaning (50 days), every 15 days the live weight and the average daily gain were determined. Data were subjected to ANCOVA considering the variable "parity".

RESULTS AND DISCUSSION

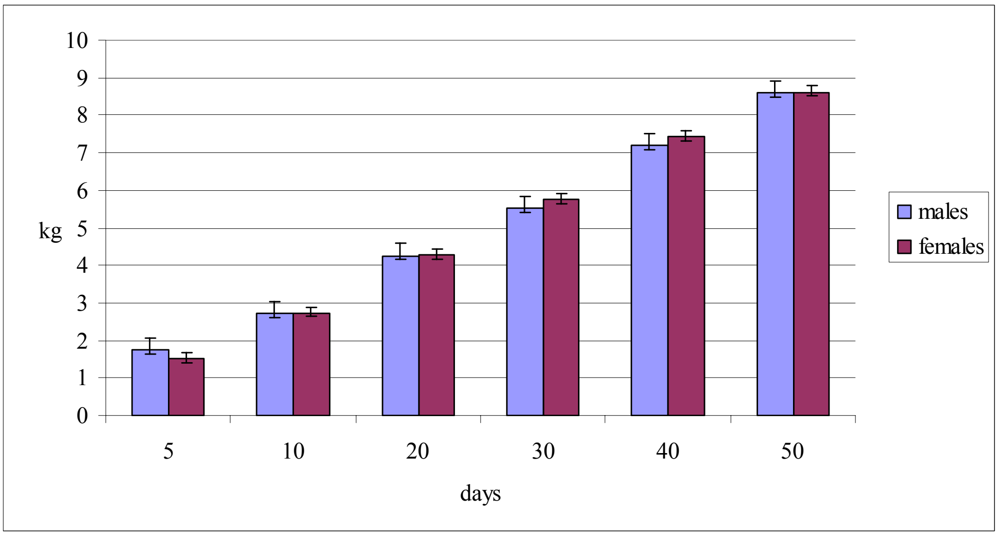
At the farrowing, the live weight of sows was 139±10 kg, according to Madonia *et al*. (2000), and the thickness of back-fat 4.38±0.32 cm. The number of piglets for each sow was 5.5±1.6, while the number of those born alive 4.8±1.0. These results are in accordance to those observed by Franci *et al* (2001) in Nero Siciliano sows reared outdoor with a number of born alive piglets of 5.2±1.9, whereas, are lower than those obtained by Matassino and Grasso (1996), in Nero delle Madonie pigs, with a number of born alive piglets of 7.7±1.4, and by Liotta *et al* (2002) in Nero Siciliano pigs with a number of born alive piglets of 7.6.

The distribution of sexes among the piglets was in favour of females with an incidence of 54% compared to that of 46% of males. The mean values of the number of teats was 10.5±0.5 for females and 11±0.8 for males therefore, all the subjects had the minimum number of 10 teats requested by the Disciplinary of the National Association of Pigs' Breeders (ANAS) and necessary for the registration to the National Pedigree Register of Nero Siciliano population.

As regards piglets, the live weight at 5 days of age was of 1.5±0.24 kg for females and 1.7±0.25 kg for males and, at the weaning, of 8.6±0.57 kg for females and 8,8±0,77 kg for males. Figure 1 reports the trend of live weight of the piglets.

The mean values of the average daily gain was 137±2.6 g/d for males and 142±2.7 g/d for females.

Figure 1. Trend of live weight male and female piglets from 5 to 50 days of age.



CONCLUSIONS

The results, however preliminary, showed that sometimes, the traditional feeding management of Nero Siciliano sows, does not allow an adequate expression of the genetic potentialities of this pig breed. Therefore is essential to adapt the nutritional strategies to the genetic evolution which the race is having in the current selection processes. Moreover, it is also interesting to pay attention to the study of the metabolic-nutritional *status* of the animals and to the characteristics of this milk, which is the main food for piglets. In fact, the knowledge of the composition of milk can provide important information for the formulation of specific diets also in the perspective of the optimization of the weaning phases (Volpelli *et al*. 1991; Liotta *et al.* 2007).

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ANALYSIS OF THE VOLATILE COMPOSITION OF FRESH AND SEASONED LARD IN "NERO SICILIANO" PIGS IN RELATION OF FATTENING DIET

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SUMMARY - The study examined the aromatic compound of lard in "Nero Siciliano" pigs, fed with acorns (group A) and barley (group B). The findings showed that fresh lard from animals of group A presented a higher percentage of alcohols (A 8.50 *vs* B 6.76; P = 0.359), terpenes (A 2.34 *vs* B 2.05; P = 0.379), aldehydes (A 18.84 *vs* B 9.22; P < 0.006), and ketones (A 7.27 *vs* B 6.71; P = 0.903), while group B showed a higher percentage of hydrocarbons (A 42.31 *vs* B 43.59; P = 0.839) and ketones (A 6.55 *vs* B 6.70; P = 0.903). Regarding the seasoned lard, in comparison with group B, group A showed a higher percentage of alcohols (A 6.80 *vs* B 3.36; P < 0.001), terpenes (A 5.79 *vs* B 4.66; P = 0.493), hydrocarbons (A 11.17 *vs* B 6.73; P = 0.051), and aldehydes (A 43.75 *vs* B 40.41; P = 0.472).

Key words: "Nero Siciliano pig", acorn, barley, volatile compound

INTRODUCTION

Dietary-induced effects on the quality traits of meat and fat are crucial to the products of "Nero Siciliano" pigs that are commonly reared in open-air systems and wooded areas. Therefore, it is important to develop a method that facilitates both the classification and the distinction of these products, with the overall objective of establishing their characteristic traits. It is well-established that the fattening diet may influence the aromatic profile of fresh and seasoned products. Therefore, the feeding strategy might be a useful factor for standardizing and identifying these products. The objective of this study was to analyze the volatile composition of fresh and seasoned lard in "Nero Siciliano" pigs that had been fed on two different diets during fattening. "Lard" intends the subcutaneous connective fat tissue lying between the retro-occipital and the backbone regions from which jowl and abdomen were removed. Today, lard from "Nero Siciliano" pigs is highly in demand, and consumers appreciate its organoleptic qualities.

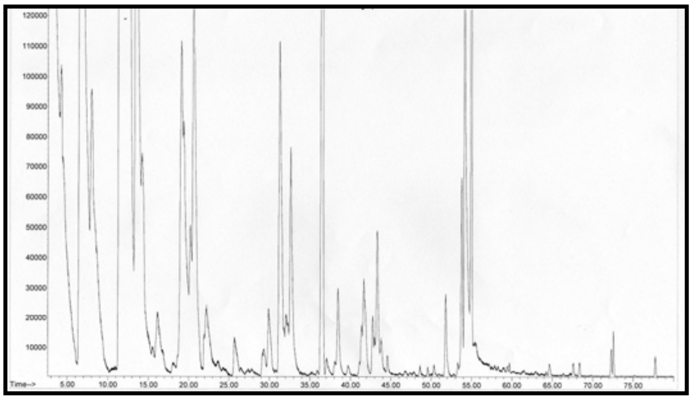
MATERIAL AND METHODS

The trial was carried out on 24 "Nero Siciliano" pigs. During the final stage of fattening, a group of 12 animals was fed with acorns (group A) whereas the other 12 were fed with barley (group B). At slaughter, a sample of lard was taken from each of the 24 carcasses. Half of each sample was cured in accordance with the dry seasoning technique used in Sicily and seasoned for 90 days. Volatile compounds of fresh and seasoned lard were extracted by headspace-SPME. Three grams of each sample were minced, weighed, placed in a 10 ml headspace vial, and sealed with a PTFE-faced silicone septum. The vial remained in a thermo block (GERSTEL MPS2) at 35°C for one hour in order to equilibrate its headspace. Then, an SPME-fibre was exposed to the headspace while maintaining the sample at 35°C. The compounds adsorbed by the fibres were identified and quantified by a gas chromatographic analysis using MS detectors. The compounds adsorbed by the fibres were desorbed from the injection port of the Agilent 6890 series gas chromatograph for six minutes at 220°C in splitless mode. The compounds were then separated in an HP-5 capillary column (J&W Scientific, 30 m, 0.25 mm i.d., film thickness 1.4 mm). The GC was equipped with an HP 5972 mass selective detector 597 *inert* (AGILENT). Helium was used as gas carrier, with a linear velocity of 27.3 cm/sec. The GC oven temperature program started when the fibre was inserted; it was kept at 38°C for 13 minutes and ramped to 110°C with an increase of 3°C/min, then to 150°C with an increase of 4°/min and to 210°C with an increase of 10°C/min, and finally kept at 210°C for five minutes. The GC-mass spectrometer interface was maintained at 240°C. Mass spectra were obtained by electron impact at 70 eV and data were collected across a range of 29-400 uma. The compounds were identified by comparison with mass spectra from a library database (Nist). The results from the volatile analyses are expressed as percentage of the total chromatographic area. The obtained data were subjected to the GLM procedure of SAS (2001).

RESULTS AND DISCUSSION

In samples of "Nero Siciliano" fresh and seasoned lard, about 80 volatile compounds (Fig. 1) were identified and assigned to the respective chemical families of aldehydes, ketones, alcohols, hydrocarbons, and terpenes.

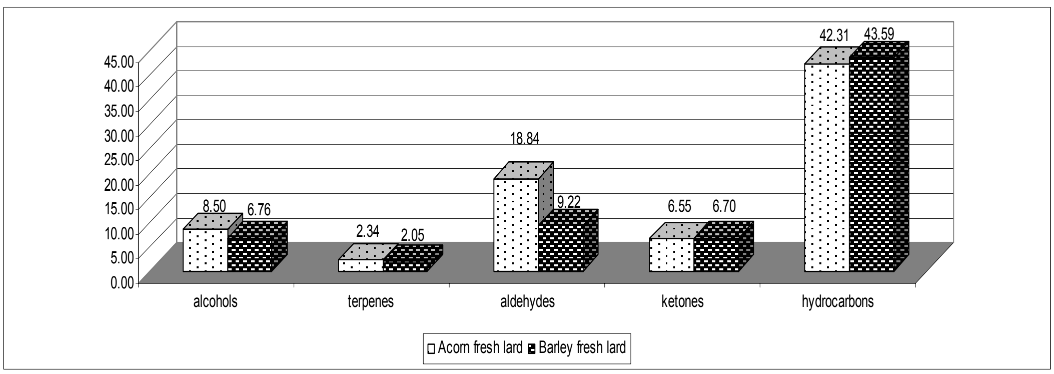
Figure1. Chromatogram of the lard.



These compounds, which are responsible for the product's aroma, generally derive from enzymatic reactions, chemical reactions, Maillard-reactions, Strecker-type degradation, and other phenomena not entirely clarified (Edwards *et al*., 1999).

The results showed (Fig. 2) that the fresh lard from pigs in group A manifested a higher percentage of alcohols (A 8.50 *vs* B 6.76; P = 0.359), terpenes (A 2.34 *vs* B 2.05; P = 0.379); aldehydes (A 18.84 *vs* B 9.22; P < 0.006), while group B showed a higher percentage of hydrocarbons (A 42.31 *vs* B 43.59; P = 0.839), and ketones (A 6.55 *vs* B 6.70; P = 0.903).

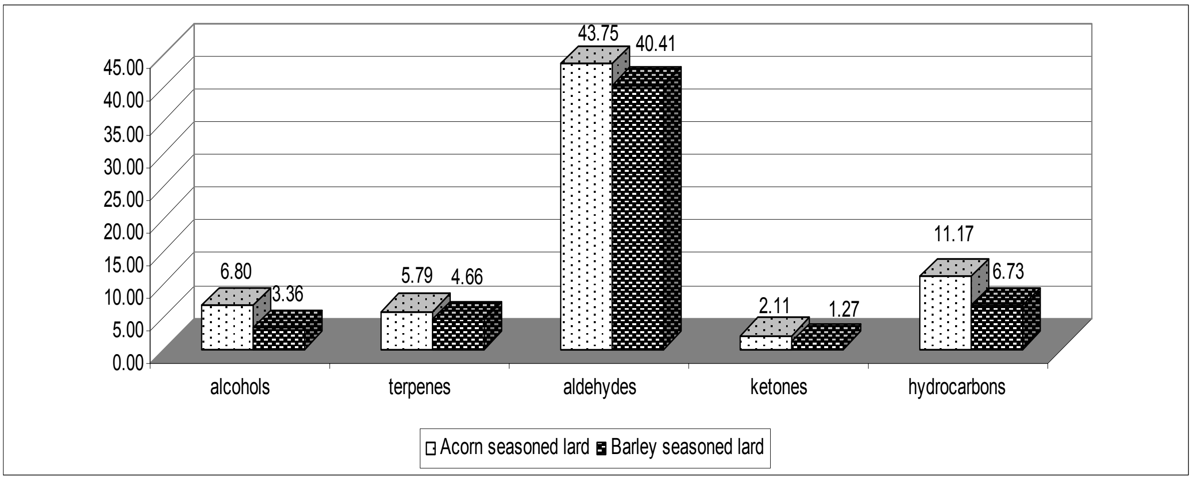
Figure 2. Major classes of aromatic compounds found in the fresh lard from pigs of both groups



In the fresh lard from both groups, hydrocarbons constituted the largest volatile group, followed by aldehydes, alcohols, and ketones. Most of these compounds derived from the autooxidation of unsaturated fatty acids. Among the aldehydes, hexanal was the most prominent in both groups. It is produced during the oxidation of unsaturated fatty acids, imparts a characteristic odour (Stahnke, 1994), and is considered a good indicator of oxidation (Shahidi and Pegg, 1994). Nonanal and 2-undecenal present in the lard were oxidation products of oleic acid, while hexanal, 2-nonenal, 2,4-nonadienal, and 2,4-decadienal were major volatile oxidation products of linoleic acid. Among the terpenes, α-pinene, δ-3-carene, limonene, α-cubebene, α-ocimene, and caryophyllene were found. These probably derived from the spices added during the processing (Moretti V.M. *et al.*,2004), or from the animals' food (Muriel *et al.*, 2004).

As for the seasoned lard (Fig. 3), aldehydes represented the predominant group of volatile compounds, followed by hydrocarbons and alcohols. In comparison with group B, group A showed a higher percentage of alcohols (A 6.80 *vs* B 3.36; $P < 0.001$), terpenes (A 5.79 *vs* B 4.66; $P = 0.493$), hydrocarbons (A 11.17 *vs* B 6.73; $P = 0.051$), ketones (A 2.11 *vs* B 1.27; $P = 0.163$), and aldehydes (A 43.75 *vs* B 40.41; $P = 0.472$).

Fig.3 Major classes of aromatic compounds found in the seasoned lard from pigs of both groups



Higher percentages of aldehydes (Nonanal, 2-undecenal, 2-propenal, pentanal, hexanal, heptanal) were found in seasoned lard from pigs fed with acorns. These results are in agreement with the ones reported by Muriel *et al.* (2004) who found elevated percentages of those aldehydes in ham from Iberian pigs that had been reared in wooded areas and fed with acorns. Moreover, in our study, furans were more prominent in pigs fed with acorns. However, in contrast to Muriel *et al.* (2004), ketones like 2-pentanone and 2,3-pentanedione also showed higher percentages in the acorn group. The conclusion which emerges from these findings is that the determination of the aromatic compound may constitute a valid instrument for establishing the product's characteristics and traceability, which is closely associated not only with the type of fattening diet, but also with the breeding system.

ACKNOWLEDGMENTS: Research financed by PRA and PRIN 2005 (Prof. Alessandro Zumbo)

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EFFECT OF FATTENING DIETS ON THE VOLATILE FLAVOUR COMPOUND OF "CINTA SENESE" SALAMI AND SEASONED LARD

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SUMMARY - The study analyzed the aromatic compound of salami and seasoned lard of "Cinta Senese" pigs that had been fed with a commercial mixture (group 1), a blend of chestnut and commercial mixture (group 2) and chestnut only (group 3). About 100 compounds from different classes were identified: aldehydes, ketones, hydrocarbons, terpenes, alcohols. The results showed that salami from group 1 contained a higher percentage of ketones (4.98%) and terpenes (42.84%). Moreover, group 2 showed a higher percentage of aldehydes (46.31%), while group 3 manifested a higher percentage of alcohols (3.89%) and hydrocarbons (17.60%). Regarding the seasoned lard, aldehydes represented the major group of the volatile compounds, followed by hydrocarbons and alcohols. A higher percentage of aldehydes (76.74%) and terpenes (3.2%) was found in group 1, while ketones (2.25%), alcohols (7.74%) and hydrocarbons (18.01%) were higher in group 3.

Key words: "Cinta Senese" , salami, seasoned lard, volatile compound

INTRODUCTION

Several old paintings, including the famous fresco called "Effetti del Buongoverno" (Effects of the good government) by Ambrogio Lorenzetti in the Palazzo Comunale at Siena, evidence the presence of the "Cinta Senese" breed in Tuscany since the 14th century. The "Cinta Senese" is the only Tuscan native pig breed that has not become extinct. Its snout is long, and it has black hair and a white belt from which the name ("Cinta") is derived. The pigs live free in the forests, eating grass and acorns. In the 1980s, due to changes in the agricultural system, the breed suffered a severe numerical reduction. Today there are about 846 sows in 153 herds. The breeding of most herds started only 10 years ago, often just as a hobby or in connection with agritourism, but increasingly also for the production of lard, salami, raw ham etc. In fact, fresh or treated "Cinta Senese" products occupy quite a solid market niche, and the elevated market value of these products resides in their special qualitative characteristics.

The aim of this research was to examine the volatile flavour compound of salami and seasoned lard of "Cinta Senese" pigs fed on different diets during fattening.

MATERIAL AND METHODS

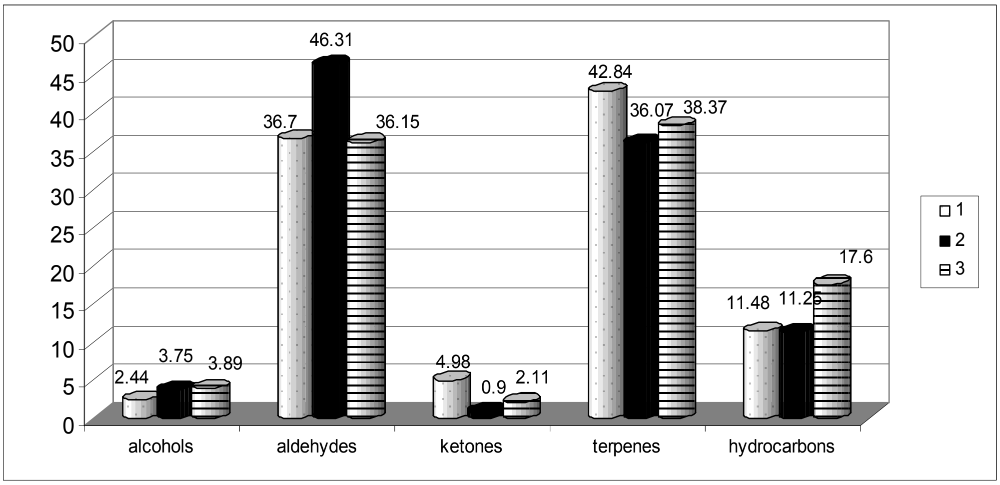
The study was carried out on 30 "Cinta Senese" pigs. During the final stage of fattening, 10 animals were fed with a commercial mixture (group 1), 10 with chestnut during the last 2 months (group 2), and the remaining 10 with chestnuts during the last 3 months (group 3). The pigs were slaughtered at an average live weight of 148 kg. At slaughtering, from each of the 30 carcasses samples of lard were taken that was subsequently seasoned (90 days) in accordance with the traditional Tuscan techniques, and from each of the three groups, salami was prepared and subjected to seasoning (45 days). Volatile compounds of seasoned lard and salami were extracted by headspace-SPME. Three grams of each sample were minced, weighed, placed in a 10 ml headspace vial, and sealed with a PTFE-faced silicone septum. The vial remained in a thermo block (GERSTEL

MPS2) at 35°C for one hour in order to equilibrate its headspace. Then, an SPME-fibre was exposed to the headspace while maintaining the sample at 35°C. The compounds adsorbed by the fibres were identified and quantified by a gas chromatographic analysis using MS detectors. The compounds adsorbed by the fibres were desorbed from the injection port of the Agilent 6890 series gas chromatograph for six minutes at 220°C in splitless mode. The compounds were then separated in an HP-5 capillary column (J&W Scientific, 30 m, 0.25 mm i.d., film thickness 1.4 mm). The GC was equipped with an HP 5972 mass selective detector 597 inert (AGILENT). Helium was used as gas carrier, with a linear velocity of 27.3 cm/sec. The GC oven temperature program started when the fibre was inserted; it was kept at 38°C for 13 minutes and ramped to 110°C with an increase of 3°C/min, then to 150°C with an increase of 4°/min and to 210°C with an increase of 10°C/min, and finally kept at 210°C for five minutes. The GC-mass spectrometer interface was maintained at 240°C. Mass spectra were obtained by electron impact at 70 eV and data were collected across a range of 29-400 uma. The compounds were identified by comparison with mass spectra from a library database (Nist). The results from the volatile analyses are expressed as percentage of the total chromatographic area. The obtained data were subjected to the GLM procedure of SAS (2001).

RESULTS AND DISCUSSION

Approximately 100 compounds of aldehydes, ketones, alcohols, hydrocarbons, and terpenes were examined for the “Cinta Senese” salami and seasoned lard. The findings showed (Fig. 1) that salami from group 1 presented a higher percentage of terpenes (42.84%) and ketones (4.98%). Moreover, group 2 showed a higher percentage of aldehydes (46.31%), while group 3 had a higher percentage of alcohols (3.89%) and hydrocarbons (17.60%).

Fig.1 Graphic representation of the major classes of aromatic compounds found in salami.

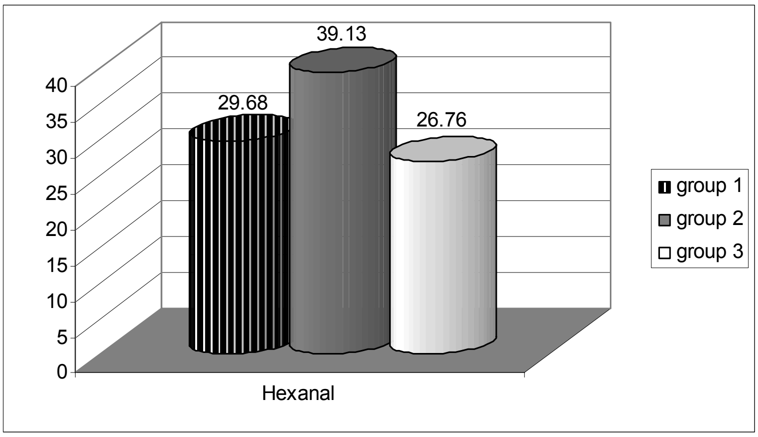


group 1 = commercial mixture
group 2 = chestnut in the last 2 months
group 3 = chestnut in the last 3 months

In order to understand the formation of flavour in salami, the origin of the volatile compounds should be identified. The flavour characteristics of seasoned salami are thought to result from a combination of microbial activities, autooxidation processes, and spices, whose relative importance varies from product to product (Edwards *et al*., 1999). Several compounds (ethanol, 3-hydroxy-2-butanone, 2,3-butanedione and 2,3-butanediol) may be formed by fermentative microorganisms via glycolysis (Kandler, 1983). Likewise, the methyl-branched aldehydes may be produced by microorganisms, from the corresponding branched chain amino acids, i.e. L-leucine, L-valine, and L-isoleucine, yielding 3-methyl butanal, 2-methyl propanal, and 2-methyl butanal respectively (Hinrichsen and Andersen, 1994). An alternative source could be a Strecker-type degradation of the same amino acids. Ethyl esters are formed enzymatically from ethanol and carboxylic acids. The ability of microorganisms to form these compounds is well established (Hosono *et al*., 1974).

However, this process seems to require a relatively long ripening time. In addition, it should be considered that aldehydes (Belitz and Grosch, 1987) and ketones (Stahnke, 1994) can be formed by well-known pathways of oxidation of unsaturated fatty acids. Also, some of the ketones and the corresponding secondary alcohols may originate in microbial β-oxidation of free fatty acids with subsequent deacylation to the β-keto acids, decarboxylation to the methyl ketone and reduction to secondary alcohols (Dartey and Kinsella, 1971). However, this might be a minor pathway because it is characteristic of moulds which are only present on the salami surface. Finally, several of the volatile compounds, such as terpenes, are well-established components of spices.To come back to our salami, the predominant aldehyde in all of the three groups (Fig. 2) was hexanal.

Figure 2 Percentage of hexanal found in salami.

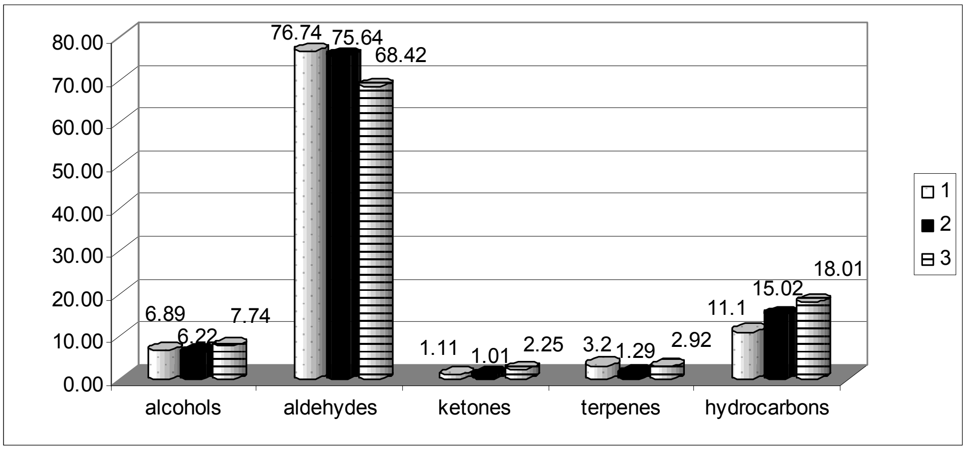


group 1 = commercial mixture
group 2 = chestnut in the last 2 months
group 3 = chestnut in the last 3 months

As already mentioned, it is produced during the oxidation of unsaturated fatty acids, imparts a characteristic green odour (Stahnke, 1994), and is considered a good indicator of oxidation (Shahidi and Pegg, 1994). Nonanal and 2-undecenal present in our salami were oxidation products of oleic acid, while hexanal, 2-nonenal, 2,4-nonadienal, and 2,4-decadienal are major volatile oxidation products of linoleic acid. In fact, oleic acid and linoleic acid constitute a large part of all unsaturated fatty acids in pork (Schlieman *et al.*, 1987). Also, the aliphatic and aromatic hydrocarbons (hexane, benzene) identified are all held to originate from the oxidation of meat lipids. Among the alcohols, ethanol, isobutanol, 1-propanol, and 1-penten-3-ol were the most common alcohols found in the salami of the three groups. Croize *et al.* (1992) found high levels of ethanol in French dry-cured sausages, but suggested that this compound was not significant for the product's aroma. On the contrary, 1-penten-3-ol is considered to give a pungent, grassy and ethereal note (Barbieri *et al.*, 1992). Concerning the terpenes, these components represent one of the most volatile group found in the "Cinta Senese" salami. In particular, α-pinene, δ-3-carene, limonene, α-cubebene, α-ocimene, and caryophyllene were found. These probably derived from the ingredients used in the spice mixture, in particular pepper. The sulphur compounds isolated, particularly diallyl sulphide and allyl methyl disulphide, derived instead from garlic which is used as an ingredient in the preparation process (Sunesen *et al.*, 2001).

As for the seasoned lard (Fig. 3), aldehydes represented the major group of the volatile compound, followed by hydrocarbons and alcohols. A higher percentage of aldehydes (76.74%) and terpenes (3.2%) was found in group 1, while the percentage of ketones (2.25%), alcohols (7.74%) and hydrocarbons (18.01%) was highest in group 3.

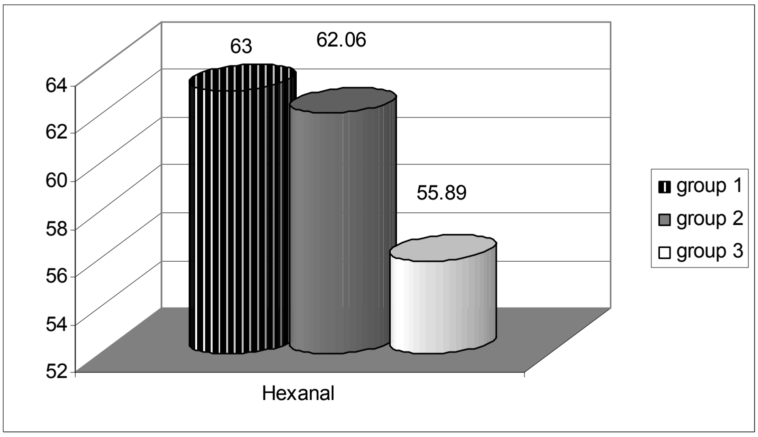
Figure 3 Graphic representation of the major classes of aromatic compounds found in lard.



group 1 = commercial mixture
group 2 = chestnut in the last 2 months
group 3 = chestnut in the last 3 months

Compared with the salami samples, the lard samples showed a higher percentage of compounds derived from oxidation of the lipid component in relation to the higher quantity of substrate present in the lard. This higher quantity could also be due to the absence of spices in the lard. Spices, in fact, have an antioxidant, lipid-protecting effect. Indeed, compared with the salami, the terpenes content (Fig. 3) of the lard appears to be extremely reduced. It should be noted that among the aldehydes, hexanal (Fig.4) is the most prominent in all of the three groups.

Figure 4 Percentage of hexanal found in seasoned lard.



group 1 = commercial mixture
group 2 = chestnut in the last 2 months
group 3 = chestnut in the last 3 months

The observation of the hexanal values shows that the lowest value, both in the lard and in the salami, is found in the samples of group 3 (pigs fed on chestnut for three months). This might be due to the antioxidant action of certain substances found in chestnuts, which might have limited the oxidation processes of the linoleic acid, a precursor of this aldehyde.

CONCLUSIONS

In view of these findings it can be hypothesized that the different lipid compositions of the three types of diet might have influenced the aromatic component of “Cinta Senese” salami and seasoned lard.

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REPLACEMENT OF SOYBEAN WITH *VICIA FABA* AND *PISUM SATIVUM* IN THE GROWHT - FATTENING OF CINTA SENESE PIG

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SUMMARY - Twenty-four Cinta Senese barrows were fed diets containing 3 different protein sources: soybean meal (S), field bean (F) and *Pisum sativum* (P) (8 pigs each group). Diets contained 14% of crude protein and 3060 Kcal/kg of Digestible Energy, as feed. Each group was reared outdoor on an area of 1 ha. In vivo performances were not different among dietary groups. At slaughter, differences in subcutaneous fat appeared only on the outer layer that had the lowest thickness in Group F. No differences were found in the sample joint composition. As regard the chemical – physical traits of meat and fat, Group S had the highest values of a* in both lean and backfat (outer layer); Group P showed higher moisture and lower fat content of meat than Group F. Field bean and *Pisum sativum* showed to be a good alternative to soybean use in the fattening of Cinta Senese pig.

Key words: Cinta Senese pig, protein source, fattening, meat quality.

INTRODUCTION

Recently, the use of soybean was forbidden in several rules regulating the pig feeding and especially in those regulations of Cinta Senese rearing (organic production, supplemented production, producers association of Cinto Toscano, producers association of Casentino ham). In fact this legume is largely genetically modified and the GMO free national product as well as being difficult to find on the market it's very expensive.

The aim of this trial was to verify the effect of use of some GMO free legumes coming from the breeding territory of the local pig as replacement of soybean.

MATERIAL AND METHODS

Twenty-four Cinta Senese pigs were reared outdoor in paddocks of 10000 m^2. Starting from 45 kg, pigs were slaughtered at 135 kg of live weight on average, after about 220 days of growing-fattening period. Animals were allotted in three groups with isoproteic and isoenergetic diets (14% CP and 3060 Kcal/kg DE) where the protein concentrate was Soybean meal (S) or Field bean (F) or *Pisum sativum* (P) (table 1).

Animals were individually weighed every 3 weeks and the feed was distributed on the basis of 90 g/kg of Metabolic Weight. Pigs of different dietary group were slaughtered all at once and some carcass measurements were recorded. Sample joint (loin from 2nd to 5th lumbar vertebra, comprehensive of surrounding backfat) was taken from the right side and dissected in the major tissues. Physical and chemical properties (chemical composition, pH, water holding capacity, Warner Bratzler shear force, colour; ASPA, 1996; Grau and Hamm, 1952; Boccard *et al.*, 1981) were determined on Longissimus Lomborum (LL) and Psoas Major (PM); colorimetric coordinates (L*, a*, b*) were measured also on the backfat. Data were analysed by GLM procedure (SAS, 2003) following different models: 1) $Y_{ijk} = A_i + bX_{ij} + E_{ij}$ (A=diet; X=initial weight) for final weight and weight gain; 2) $Y_{ijk} = A_i + B_j + bX_{ijk} + E_{ijk}$ (A=diet; B=day of slaughter; X=slaughter weight) for carcass and sample joint composition and for physical traits of muscle; 3) $Y_{ijkl} = A_i + B_j + C_k + bX_{ijkl} + E_{ijkl}$ (A=diet; B=day of slaughter; C=muscle (LL, PM); X=slaughtering weight) for chemical composition of muscle.

Table 1. Composition of diets.

		Diet		
		S	F	P
Maize	%	35	20	14
Barley	%	40	45	42
Soybean meal	%	12	-	-
Field bean	%	-	22	-
Pisum sativum	%	-	-	31
Wheat bran	%	12	12	12
Vitaminic premix	%	1	1	1

RESULTS AND DISCUSSION

Regarding in vivo performances (table 2) no significant differences were found. ADG was similar for all the groups and consequently, animals fed different diets could reach the same slaughtering weight. Also the carcass characteristics were similar except for thickness of backfat (in its outer layer at last thoracic vertebra) which was lower for group F. No significant difference was found among dietary groups for tissue composition of sample joint (table 3).

Table 2. Effect of diets on *in vivo* performance and carcass traits.

		Diet			
		S	F	P	RSD
Initial live weight	kg	45.25	44.31	44.12	10.54
Final live weight	kg	132.5	136.8	138.8	9.12
ADG	kg	0.403	0.423	0.433	0.05
Dressing percentage	%	80.85	80.52	80.41	2.16
Backfat thickness					
GM (total)	cm	4.90	4.49	4.76	0.70
- Outer layer	cm	2.19	2.31	2.31	0.45
- Inner layer	cm	2.71	2.18	2.45	0.56
LT (total)	cm	4.99	4.63	4.97	0.59
- Outer layer	cm	2.17 a	1.61 b	2.28 a	0.53
- Inner layer	cm	2.83	3.02	2.68	0.55

a, b = P<0.05

Table 3. Effect of diets on sample joint composition

		Diet			
		S	F	P	RSD
Sample joint	kg	2.076	2.119	2.100	0.173
Subcutaneous fat (total)	%	44.69	46.61	46.89	2.63
- Outer layer	%	18.60	19.72	18.77	1.94
- Inner layer	%	26.09	26.89	28.12	2.89
Intermuscular fat	%	6.08	4.64	5.99	1.91
Lean (total)	%	39.39	39.91	38.45	2.85
- *Poas major*	%	8.16	8.48	7.38	0.88
- *L. lumborum*	%	25.33	25.89	25.92	2.38
- Other lean	%	5.90	5.54	5.15	0.98
Bone	%	9.48	8.85	8.68	1.60

Concerning physical traits of LL muscle (table 4), differences were found only for a* colour parameter: pigs of S dietary group showed the most red meat. Moreover, the same S group showed the highest value of a* parameter also on the outer layer of backfat.

Different protein source affected the chemical composition of the two muscles (table 5): group F supplied meat with higher moisture and lower ether extract content than group P. As regard to the difference between muscles, LL was less moist and fatter than PM.

Table 4. Effect of diets on physical traits of meat and fat

			Diet			
			S	F	P	RSD
Longissimus Lumborum:						
- Drip loss		%	2.17	1.84	1.77	1.34
- Cooking loss		%	25.56	24.45	24.98	2.91
- Free water		cm^2	11.1	12.5	11.9	1.49
- Shear force on raw meat		kg	7.70	8.24	7.63	1.50
- Shear force on cooked meat		kg	10.21	11.06	9.54	3.31
- pH45min			6.60	6.44	6.50	0.25
- pH24h			5.77	5.88	5.69	0.16
- L*			46.49	46.08	48.28	2.69
- a*			12.24 a	10.41 b	10.88 b	1.22
- b*			3.69	2.95	3.93	1.28
Outer layer backfat:	- L*		80.95	81.53	81.88	0.90
	- a*		3.10 a	2.61 b	1.89 b	0.68
	- b*		2.95	3.03	2.74	0.43
Inner layer backfat:	- L*		83.16	82.78	83.13	0.91
	- a*		2.43	2.59	2.05	0.62
	- b*		2.57	2.90	2.54	0.58

a, b = P<0.05

Table 5. Effect of diets on chemical traits of muscles (*L. lumborum* and *Psoas Major*)

		Diet			Muscle		RSD
		S	F	P	LL	PM	
Moisture	%	72.25 ab	72.74 a	72.06 b	71.44 a	73.26 b	0.75
Protein	%	21.90	21.84	21.61	21.97	21.56	0.72
Ether Extract	%	4.21 ab	3.75 a	4.71 b	5.10 a	3.37 b	0.97
Ash	%	1.12	1.15	1.24	1.12	1.22	0.17

a, b = P<0.05

CONCLUSIONS

The uniformity of the results with the tested diets indicates that Field bean and *Pisum sativum* could be a good alternative to soybean meal in fattening of pig in outdoor breeding system. These protein sources could be used in fattening and finishing periods of Cinta Senese pigs to obtain balanced diets. Moreover, their use is desirable for supplementation of pasture in wood, as both acorn and chestnut are well-known as poor in protein content (Sirtori *et al.*, 2005; Acciaioli and Pianaccioli, 2004).

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PRODUCTION TRAITS OF BLACK SLAVONIAN PIGS

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SUMMARY - Black Slavonian pig is an autochthonous Croatian breed created in the second half of the 19th century. Until the 1950s it was the most widespread breed in the Slavonia, mainly used for the production of fat and meat products. Recently, the population was drastically reduced and in 1990s the survival of the breed was endangered. Due to current protection measures the effective population rather increased; in 2006 there were 46 boars and 604 sows. The breed is well adopted for outdoor keeping. Traditional production includes the utilization of pasture and woodland with supplement of a small amount of grains. Litter size of the breed is low, on average 7-8 piglets. The fattening abilities are also modest, with low daily gains and high share of fat in the carcass (>40%). However, the meat quality is good, with high content of intramuscular fat (6-7%) and high appreciation of its meat products.
Key words: pigs, breeds, Black Slavonian pig, production traits.

ORIGIN AND POPULATION

Black Slavonian pig (Photo 1) is an autochthonous Croatian breed which originated from Berkshire and Poland China breeds crossed with Black Mangalitsa. The breed was developed in the east of Croatia (Slavonia region) by Count Pfeiffer at the end of the 19th century. In the past, the breed was commonly used for production of traditional meat products, like famous Slavonian Kulen, a slowly fermented and dried spicy sausage stuffed in pork blind gut. Nowadays, this breed is rare and meat products are produced mainly from the meat of modern white breeds and crosses.

Photo 1. Black Slavonian pig.

By importing thoroughbred pig breeds, the population of Black Slavonian Pig was drastically reduced. In 1996, the size of effective population was less than 20 and survival of the breed was endangered (Uremović *et al*., 2000). The same year, Croatia signed the Biodiversity Treaty (CBD, 1992) and ¨A Survey of the State of Biological and Environmental Diversity of Croatia with Strategy and Protection Plan Action¨ was elaborated (DUZZP RH, 1999), as well as ¨A Program for Breeding up of the Black Slavonian Breed¨(Uremović and Janeš, 2000). As a result of undertaken measures and state subsidies, a reduction in the number of Black Slavonian Breed was stopped. In recent years effective population rather increased, in 2006, the number of boars was 46, young boars 12, sows 604 and young sows 185 (Croatian Livestock Center, 2007). However, the genetic diversity of the breed is reduced (Bradić *et al.*, 2007).

TRADITIONAL PRODUCTION SYSTEM

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

PRODUCTION TRAITS

Production traits of Black Slavonian breed have not been sufficiently investigated. However, as for most of the local autochthonous breeds the production traits are rather limited. Uremović *at al*. (2000) found that average number of live born and reared piglets per litter was 6.89 and 5.76, respectively. The fattening abilities are also modest. Under extensive conditions of fattening, daily gain in body mass starting at 27.20 kg and reaching 106.05 kg was 478 g, with low meat percentage in the carcass (42.95 %) (Uremović *et al*., 2000). In a few previous papers, even lower meatiness in the carcasses of similar weight (~80 kg) was reported: 28.59 % (Petričević *et al*., 1988) and 28.51 % (Kralik *et al*., 1988). In general, Black Slavonian pigs are characterized by high proportion of adipose tissue in the carcass. For example, in heavy Black Slavonian fatteners (in the age of about 18 months and average body weight of 160 kg) used for the processing of Slavonian Kulen sausage, the average depth of meat and fat above *m.gluteus* were nearly the same, 64 and 63 mm, respectively. While in the carcasses of crossed white pigs (Large White x Swedish Landrace sired with Duroc) of similar age and weight the average depth of meat and fat were 73 and 30 mm, respectively. As a consequence, in comparison to modern white crosses, Black Slavonian pigs have significantly lower utilization of primal cuts (hams, back, shoulders and neck) (32.33 vs. 26.75 %, $P<0.05$) and lower utilization of carcass (19.85 vs.16.26 %, $P<0.05$) for the production of traditional products like Slavonian Kulen (Karolyi *et al*., 2004). These results however, were obtained after the prolonged period of pre-slaughter fattening in the piggery when weight gain of pigs is mostly due to accumulation of fat. Results from traditional outdoor-low input system, reported by Senčić *et a*l. (2005), showed practically the same shares of fat and muscle tissue (40.96 and 41.00 %, respectively) in the carcasses of Black Slavonian pigs reared until 12 months of age and average body weight of 130 kg. In the same experiment, significant improvements in the carcass meatiness were obtained by crossing Black Slavonian pig with Swedish Landrace boars (in F_1 progeny the share of fat and muscle were 36.03 and 44.59 %, respectively).

WELFARE IN THE TRADITIONAL PRODUCTION

The knowledge about welfare issues in Black Slavonian breed is limited. No research based on standard scientific measurements of animal welfare (Broom, 1992) has been preformed. However, it could be expected that in the traditional outdoor systems, both physical and mental well-being of the animals are good. The pigs are reared extensively, they spend most of their life-time outside on pastures and oak woods where they are able to exhibit foraging behaviour and other natural instincts. In addition, the interaction between animals and humans is normally positive as animals are usually very confident in herd keepers and most herdsmen respect their own animals. If coping with environment is easy, the animal welfare is normally good (Broom, 1992).

Parasite and other diseases

It is well known, however, that organic and free-range livestock farming is more often associated with parasite-related diseases (Lund and Algers, 2003; Hovi *et al*., 2003). One of the recent no-drug recommendation methods of gastrointestinal nematode control in grazing animals is the consumption of plants rich in condensed tannin, as it has a direct toxic effect on parasites and/or on the parasite fecundity (Hoskin *et al*., 2000). Forages rich in condensed tannin have been found to improve performance of animals that had high faecal parasite egg counts (Niezen *et al*., 1998), reduce egg output and worm burden in general (Butter *et al*., 2000). By grazing in oak woods, especially during season when alternative forage availability is scarce, Black Slavonian pigs may consume huge amounts of tannin rich plant material. To research tannin protective ability, Salajpal *et al*. (2004) fed Black Slavonian pigs three weeks before slaughter with acorn *ad libitum* (experimental group) or with concentrated feed (control). All examined pigs were reared (spring-autumn) on pasture utilizing natural resources and they naturally acquired parasite. It was founded that oak acorn (*Quercus robur L.*) is a relatively tannin rich forage (65 gkg^{-1} of DM) and that its consumption can reduce total faecal egg count output (96.01 %) in pigs infected with large roundworm (*Ascaris suum*) and other gastrointestinal parasites. It is concluded that the acorn grazing in the traditional Black Slavonian pig production system may have a potential of aiding in the control of the gastrointestinal parasites and consequently may result in reduced need for anthelmintic treatment.

The major potential danger in pig production in East parts of Croatia currently is the swine pest threat. It is now a second year since the vaccination against infection has been banned, according to EU recommendation and outbreaks of swine pest have been reported recently in piggeries in villages of borderland area at the East of Croatia. However, the disease has not occurred in the populations of Black Slavonian pigs and the sanitation of infected piggeries was successful.

Transport, handling and pre-slaughter practice

With winter, the season of pig slaughtering approaches and traditional meat processing begins. In the past, the slaughtering typically took place in the backyards; today pigs are slaughtered in the slaughter-houses. The pigs are transported to slaughter-house mostly by trucks or, if the distance to slaughterhouse is short by tractors basket (max 4-5 animals).There is no data about influence of loading-unloading or transport density or duration on welfare of Black Slavonian pigs. Slaughtering practice for Black Slavonian pigs is the same as for any other pigs in the commercial abattoirs. In Croatia pigs are predominantly stunned by the use of electricity, while exposure to CO_2 is in usage in a few abattoirs outside of Slavonia region. The lairage time may differ from virtually zero when pigs going to the slaughter line immediately after they were unloaded from truck (what is common for local small-scale plants in the winter season) to couple of hours or longer, even overnight in the larger slaughterhouses with big livestock stores. Water, but not feed are usually provided during longer pre-slaughter resting. However, it is still not uncommon that those responsible for handling of animals during transport or in the slaughterhouses tend to treat the animals in an inconsiderate way.

Transport and handling usually include removal from the familiar fattening pen, loading, transport, unloading, mixing with unfamiliar pigs, overcrowding, interaction with slaughterhouse personnel and exposure to novel environments and conditions. All these practices, as it is known, may impose stress which impairs both animal welfare and meat quality (Broom, 1993, Lambooij and van Putten, 1993, Warris *et. al*., 1998). Physiological responses to stress in pigs can be assessed by measuring a changes in plasma cortisol, glucose, lactate and/or other parameters like activity of the cellular enzymes creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) which are commonly used as indicators of stress affecting muscle damage (Fàbrega, 2002, Perez *et. al*., 2002). There are some indications that the resistance to stress during pre-slaughter handling of Black Slavonian pigs seems to be superior to modern pigs. In the comparison with modern white crosses, Black Slavonian pigs had the lowest (P<0.05) serum CPK and AST activity in the blood samples collected at exsanguinations, which may indicate their lower susceptibility to pre-slaughter stress (Karolyi *et al*., 2004). Nevertheless, this single finding needs to be investigated in the future in more detail (e.g. cortisol level).

MEAT QUALITY

Pre-slaughter stressors can also change muscle glycogen metabolism and affect meat colour and water holding capacity (WHC) (Gispert *et al*., 2000). Short pre-slaughter stress may cause a rapid fall in muscle pH *post mortem* and development of pale, soft and exudative (PSE) meat in stress susceptible animals. Long term stressors, however, may cause muscle fatigue and depletion of muscle glycogen reserve, producing dark, firm and dry (DFD) meat after slaughter. Regarding meat quality traits, no prominent defects in the meat from Black Slavonian pigs were reported. The pH_i and pH_u values of *m.longissimus dorsi* were inside normal scope and ranged between 6.11-6.60 and 5.80-5.87, respectively (Karolyi *et a*l., 2004, Senčić *et al*., 2005; Karolyi *et al*., 2007). The meat of Black Slavonian pigs is visually darker and redder than the meat from modern pigs. The lightness (Cie *L**) and redness (Cie *a**) values of longissimus muscle colour were 49.93 and 20.02, respectively (Karolyi *et al*., 2004). The water fixation ability was 4.50 cm^2 (Senčić *et al*., 2005). The most distinctive characteristic of meat of Black Slavonian breed in comparison to pork from modern pig breeds is particularly high content of intramuscular fat (IMF), in average 6 to 7 % (Uremović *et al*., 2004; Senčić *et al*., 2005; Karolyi *et al*., 2007). Intramuscular fat influences juiciness, flavour, tenderness and visual characteristics of meat (Miller, 2002). Optimal level of IMF for pork is generally considered between 2.5-3.0 % (Grebens, 2004), while low IMF content may impair chewing properties of meat. However, excessively high content of IMF (>6 %) make fat in the meat become too visible which may avert the consumer (Resurreccion, 2003; Miller, 2002).

Consumers' attitudes and preferences

The consumer attitudes toward fresh meat from Black Slavonina pigs have not been investigated yet. In general, Croatian consumers consider organic products as very healthy, of good quality and tasty (Radman, 2005a). Meat and products from traditional breeds, like Black Slavonian pig, have a good image in the public and media. They are perceived as better and of higher quality than meat and products of modern breeds. Surprisingly, when consumers' preferences for Slavonian Kulen made from Black Slavonian or modern pigs were investigated by means of a blind sensory test the acceptance of the kulen produced from Black Slavonian pigs by respondents (n=248) was the lowest (Radman *et al*., 2005b). These results however, could be partly due to a variable quality of artisan meat products whose sensory characteristics may differ among producers, localities and seasons. Therefore, the research should be repeated with more products from different producers in order to have more reliable indications about consumers' preferences of Black Slavonian pig's meat products.

The human health issue

It is well known that industrial pork in general has unacceptably high ratio of n-6 and n-3 polyunsaturated fatty acids (Wood *et a*l., 2003). The n-6/n-3 index is particularly high if animals were intensively fed with concentrated feeds, because the cereals are rich in linoleic acid (C18:2n-6). The influence of feedstuff in the traditional feeding system of Black Slavonian pigs, which includes utilization of the natural resources of pasture and oak, on fatty acid profile of meat has been investigated by Karolyi *at al*. (2007). The indication of beneficial effects of oak (*Quercus robur* L.) was found when Black Slavonian pigs were fed with acorn or with concentrate feed during pre-slaughter fattening. Feeding acorn *ad libitum* three weeks before slaughter significantly increased the content of alpha linolenic acid (C18:3 n-3) in the longissimus muscle in comparison to concentrate fed group (0.37 vs. 0.12, respectively; expressed as % of total fatty acid methyl esters, P<0.01). As consequence, the n-6/n-3 ratio in the muscle of acorn finishing pigs was nearly threefold lower than in concentrate finishing pigs (24.1 vs. 69.3, $P \leq 0.01$).

CONCLUSIONS AND FUTURE WORK

It could be concluded that *in-situ* conservation of local Black Slavonian breed so far has been successful. Currently, the main potential risk for breed's future is a swine pest threat. However, the long term re-establishment of the population of Black Slavonian breed must be considered on economical basis. As a part of current trends of support for sustainable and traditional food production systems, it becomes important to preserve the traditional production of meat products from local breeds. In this way, it is reasonable to start breeding the Black Slavonian pigs again for the production of Slavonian Kulen sausage and other value added artisan meat products. This could be, by itself, the best way for long-term preservation of Black Slavonian breed. For improvement of some production traits, crossing with more productive breeds (e.g. Swedish Landrace or Duroc) may be considered.

Regarding to welfare of Black Slavonian pigs all positive aspects of traditional breeding system need to be preserved and those potentially harmful, like poor slaughterhouse practices, should be changed or improved. However, the basic investigations on animal welfare are needed, because there is a lack of scientific data which can be used for objective assessments of stress and consequently for improvements or control of welfare. In general, the understanding of importance of good animal handling must be improved among transport and slaughterhouse workers who handle animals. To control this improvement and animal welfare in the whole production chain, the reliable system of control needs to be established.

Additional research is needed on muscle quality profiling, as well as fat quality. The most interesting muscle trait, the high content of intramuscular fat, should be related, both to sensory and nutritional properties of meat. The fatty acid profile of meat should be researched in detail with special emphasis on influence of traditional feedstuffs (i.e. pasture and acorn) on profile of fatty acids in the meat and potential benefits on consumer health. If there are such benefits, they must be promoted in the public and consumers must be informed.

Increase in production of traditional processed meat products is crucial for breed preservation, as it is already pointed. However, the standardization of technology must provide more uniformity of product traits. In general, the product traits need to be scientifically characterized in more detail in order that quality can be defined and objectively controlled in the future. Consumer preferences for meat and products of Black Slavonian breed also needs to be additionally investigated.

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GROWTH PERFORMANCE AND MEAT QUALITY OF OUTDOOR REARED CALABRESE PIGS

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SUMMARY - Many chain agreements and regulations for products protected by PDO and PGI names include a ban on raw materials and feed obtained from genetically modified organisms (GMOs) for the protection of the consumer. Furthermore, the prohibition against the use of meat protein in the farming of income-generating animals has given rise to an increasing need for alternative sources of protein which are economically compatible with farm production costs.
In recent years Regional Agency for Agricultural Development Services (ARSSA) of Calabria Region has been promoting outdoor pig production in order to increase the number of pig farms in the region and to develop the chain of traditional products including, among others, the products obtained from pigs of *Calabrese* breed. The main aim of this study was to compare different GMO-free diets for *Calabrese* pigs using local feed with an assessment of the main indicators of on-farm and slaughter performance.

Key words: pig feed, GMO, pork quality, *Calabrese* breed.

INTRODUCTION

In recent years the Demonstrative Experimental Centre for the "Safeguarding and development of the black *Calabrese* pig" of the Regional Agency for Agricultural Development Services (ARSSA) of Calabria Region has been promoting outdoor pig production in order to increase the number of farms in Calabria and to strengthen the traditional products production chain with particular emphasis on those obtained from the pigs of *Calabrese* breed. The Agency has identified the need to obtain a better understanding of related production performance, in particular with regard to growth and slaughtering of pigs of this breed.

The formulation of livestock feed has always been strongly influenced by availability and hence the cost, of soya. In effect, out of world production of grain legumes of about 240 million tons, about 75% is represented by soya.

Much interest in Europe however, has been given to alternative raw materials such as the proteic pea, field beans and lupines.

In reality, the needs of the European feed industry for proteic raw materials are substantially in excess of production, giving rise to a negative balance of 74% (ASS.I.CA, 2007).

The situation in Italy is on average the same as that for Europe even though there are significant differences. Italy is a marginal producer of the pea although in Europe as a whole, this species represents 84% of grain legume production. Italy contributes 75% of European soya production – these quantities are still significantly less than internal needs. It also accounts for 45% of European production of alfalfa.

Recent increases in the attention given to "local and environmental background" of protected typical products and those obtained with organic methods has forced the industry to make the best possible use of other agricultural products in livestock farming, seeking to maximise and optimise their inclusion in the diet of the animals concerned.

To this is added the increasing need to guarantee quality chains with the exclusion of feed raw materials obtained from genetically modified organisms (GMOs) with the aim of preserving the special nature of the product and safety for the consumer. A specific legislative restriction of this kind is permitted by EC Reg. 50/2000 and EC Reg. 1830/2003.

The first relates to the labelling of feed products and ingredients containing genetically modified additives and flavourings while the second is concerned with the traceability and labelling of GMO foods and feeds.

In this context it is also important not to undervalue the importance of trends in the prices of grain, starch products and animal feed. These experienced sharp rises over 2006 as compared with 2005, equal to 11.8% and 5.6% respectively (ASS.I.CA 2007).

The general objective of this study is the development of a chain in Calabria for the production of a non-GMO pork obtained from the *Calabrese* pig with the elimination of raw materials which may potentially be genetically modified (soya in particular). This may be achieved through the use of proteinaceous crops other than soya produced locally with tests on the consequential effects on on-farm performance and the quality of the carcases and pork (Gentry *et al.* 2001; Gentry *et al.* 2004).

The activities involved come within the *National Plan for the production of Vegetable proteins*, aiming to meet at least a part of Italian requirements, to offer non-GMO products (for which there is a substantial increase in demand) and to enhance the related production chains.

MATERIAL AND METHODS

An experimental trial was carried out involving the outdoor production of pigs fed with non-GMO vegetable feed compared with pigs fed with a conventional soya-based diet. The trial was carried out at the Demonstrative Experimental Centre for the "Safeguarding and development of the black *Calabrese* pig" near Acri, in the Province of Cosenza at a site which was about 700 metres above sea level. Two groups of 36 *Calabrese* pigs were used and fed on two different diets, one based on soya and the other based on proteinaceous crops produced locally.The animals were housed in three enclosures per group, each of a size to accommodate 12 animals. Each enclosure was rectangular in form and situated on flat land, had a surface area of 12,000 square metres (100 square metres per head) including feeding troughs, drinking troughs and insulated mobile shelters.

The animals, born during the week from 19 to 25 September 2005, were all introduced into the enclosures on the same day. They were fed ad libitum with isoproteic and isoenergetic diets (tables 1, 2, 3, 4, 5 and 6). The live-weight of all the pigs in the trial was measured (initial, at the end of the growing stage and at the end) as was the consumption of feed per pen in order to establish daily weight increase and the feed conversion index. Each animal was slaughtered at different ages but in pairs for the two groups – two enclosures at 9 months, 2 enclosures at 10 months and 2 at 11 months.

Table 1. Feed Ingredients – growing (% of whole)

	No soya	Soya
Digestible energy (ED), kcal/kg	3.506	3.492
Humidity	12.3	11.8
Lysine	0.75	0.77
Raw protein	15.4	15.5
Raw lipids	2.2	2.4
Raw fibre	7.0	4.9
Ashes	5.3	5.6

Table 2. Food ingredients – fattening (% of whole)

	No soya	Soya
Digestible Energy (KCal/kg)	3,596	3,620
Humidity	11.9	12.6
Lysine	0.64	0.64
Raw Protein	14.2	14.2
Raw lipids	2.1	2.8
Raw Fibre	4.6	3.9
Ashes	5.1	5.2

Table 3. No Soya diet, % dry matter (30 to 70 kg live weight)

RAW MATERIAL	% of whole	% Dry matter	R.P.	Ashes	N.E.	N.E.	R.L
Acri Maize	34.05	85.2	9.74	2.17	81.22	2.64	4.23
Acri soft wheat	14.50	88.8	13.29	2.14	79.56	3.26	1.75
Acri barley	14.50	90.0	11.61	2.58	78.33	5.39	2.09
Sunflower solvent	14.10	90.0	32.78	6.91	28.87	29.44	2.00
Brown field beans	13.00	87.0	30.34	3.89	55.66	8.62	1.49
Acri P. Pea	7.00	86.2	27.26	3.70	62.80	6.10	1.50
Calcium carbonate	1.20	99.8	0.0	100.0	0.0	0.0	0.0
Bicalcium phosphate	1.20	95.0	0.0	86.32	0.0	0.0	0.0
Sodium Chloride	0.31	99.8	0.0	100.0	0.0	0.0	0.0
L-Lysine	0.12	98.0	97.55	0.0	0.0	0.0	0.0
Copper sulphate	0.01	71.0	0.0	88.73	0.0	0.0	0.0
Zinc oxide	0.01	99.8	0.0	96.99	0.0	0.0	0.0

R.P: Raw protein, N.E.: Nitrogen-free extracts, R.F.: Raw fibre, R.L.: Raw lipids.

Table 4. Soya diet, % dry matter (30 to 70 kg live weight)

RAW MATERIAL	Feed %	% Dry matter	R.P.	Ashes	N.E.	R.F.	R.L.
Acri Maize	22.71	85.2	9.74	2.17	81.22	2.64	4.23
Acri soft wheat	21.50	88.8	13.29	2.14	79.56	3.26	1.75
Acri Barley	21.50	90.0	11.61	2.58	78.33	5.39	2.09
Soft wheat bran	18.00	88.00	17.44	5.74	61.83	10.63	4.36
Soya solvent	13.30	87.50	49.94	7.43	32.69	8.57	1.37
Calcium carbonate	1.20	99.8	0.0	100.0	0.0	0.0	0.0
Bicalcium phosphate	1.20	95.0	0.0	86.32	0.0	0.0	0.0
Sodium Chloride	0.35	99.8	0.0	100.0	0.0	0.0	0.0
L-Lysine	0.22	98.0	97.55	0.0	0.0	0.0	0.0
Copper sulphate	0.01	71.0	0.0	88.73	0.0	0.0	0.0
Zinc oxide	0.01	99.8	0.0	96.99	0.0	0.0	0.0

At slaughtering specially trained staff identified a series of quality parameters (EC, 2001; Novelli *et al.* 1991):

- carcase meat to fat ratio (using a Fat-O-Meater);
- carcase weight;
- weight of lean and fat cuts;
- thickness of dorsal fat cover at the level of the gluteus medius muscle, at the caudal, mid and cranial position, maximum at the shoulder and average on the back;
- the meat pH with a penetrating pH-reader, taken at 45 minutes and 24 hours after death at the thigh muscles (semi-membranous muscle and the loin (the *longissimus dorsi* muscle).

Table 5. No Soya Diet, % dry matter (70 to 130 kg)

Raw material	Feed %	% Dry matter	R.P.	Ashes	N.E.	R.F.	R.L.
Acri soft wheat	42.27	88.8	13.29	2.14	79.56	3.26	1.75
Acri barley	7.00	90.0	11.61	2.58	78.33	5.39	2.09
Brown field bean	13.00	87.0	30.34	3.89	55.66	8.62	1.49
Soft wheat bran	15.00	88.00	17.44	5.74	61.83	10.63	4.36
Acri Maize	13.00	85.2	9.74	2.17	81.22	2.64	4.23
Acri P. Pea	7.00	86.2	27.26	3.70	62.80	6.10	1.50
Bicalcium Phosphate	1.10	95.0	0.0	86.32	0.0	0.0	0.0
Sodium Chloride	0.35	99.8	0.0	100.0	0.0	0.0	0.0
L-Lysine	0.06	98.0	97.55	0.0	0.0	0.0	0.0
Copper sulphate	0.01	71.0	0.0	88.73	0.0	0.0	0.0
Zinc oxide	0.01	99.8	0.0	96.99	0.0	0.0	0.0
Calcium carbonate	1.20	99.8	0.0	100.0	0.0	0.0	0.0

Table 6. Soya diet, % dry matter (70 to 130 kg)

Raw material	Feed %	% Dry matter	R.P.	Ashes	N.E.	R.F.	R.L.
Acri Maize	49.45	85.2	9.74	2.17	81.22	2.64	4.23
Acri barley	15.00	90.0	11.61	2.58	78.33	5.39	2.09
Soya Solvent	12.70	89.50	49.94	7.43	32.69	8.57	1.37
Soft wheat bran	10.00	88.00	17.44	5.74	61.83	10.63	4.36
Acri soft wheat	10.00	88.8	13.29	2.14	79.56	3.26	1.75
Bicalcium phosphate	1.40	95.0	0.0	86.32	0.0	0.0	0.0
Calcium carbonate	1.00	99.8	0.0	100.0	0.0	0.0	0.0
Sodium chloride	0.33	99.8	0.0	100.0	0.0	0.0	0.0
L-Lysine	0.10	98.0	97.55	0.0	0.0	0.0	0.0
Copper sulphate	0.01	71.0	0.0	88.73	0.0	0.0	0.0
Zinc oxide	0.01	99.8	0.0	96.99	0.0	0.0	0.0

Meteorological data were recorded during the rearing period relating to temperature, humidity and rainfall by the ARSSA Agro-meteorological Service of Service of Villa Santa Margherita (KR).

The data collected over the trial have been processed statistically by the Research Centre on Animal Production (CRPA Spa) with variance analysis, the Anova test and Duncan test using the SPSS for Windows software package.

RESULTS AND DISCUSSION

Farming

The tables set out below contain the farming production indices divided into the different stages (Tables 7, 8 and 9) as well as the overall results (table 10 and 11).

In the first stage (table 7) it is clear that the two trial groups began with significantly different weight per animal (P<.01), a difference which becomes more marked at the end of the stage. Thus the "no-soya" group had a better daily weight increase than the "soya" group (p<0.01). There is however, no difference with regard to feed consumption and the feed consumption index.

Table 7. On-farm performance A (February to March)

	No Soya	Soya
Pens, n.	3	3
Animals, n.	36	36
Weight 1 pen, kg	482.9 ± 134.9	405.3 ± 120.6
Weight 1 animal, kg	40.2 ± 9.5 A	33.8 ± 8.7 B
Weight 2 pen, kg	866.7 ± 96.7	752.3 ± 105.9
Weight 2 animal, kg	72.2 ± 7.8 A	62.7 ± 8.6 B
Daily Weight Index 1 animal, g	769.1 ±89.9 A	697.8 ± 94.5 B
Consumption 1 pen, kg	1,144.3 ± 74.2	1,023.3 ± 108.2
Consumption 1 animal/day, kg	2.3 ± 0.3	2.1 ± 0.3
Feed conversion rate 1 pen	3.0 ± 0.3	3.0 ± 0.2

Different letters indicate differences for p<0.01

Table 8. On-farm performance Stage II (March to May)

	No Soya	Soya
Pens n.	3	3
Animals, n.	36	35
Weight 3 pen, kg	1,382.6 ± 84.2	1,229.3 ± 147.0
Weight 3 animal, kg	115.2 ± 9.2 A	105.4 ± 9.7 B
Daily Weight Index 2 animal, g	767.1 ± 107.5	757.0 ± 113.8
Consumption 2 pen, kg	2,193.2 ± 70.0	2,192.7 ± 55.9
Consumption 2 animal/day, kg	3.3 ± 0.1	3.3 ± 0.1
Feed conversion rate 2 pen	4.3 ± 0.3	4.5 ± 0.2

Different letters indicate differences for p<0.01

Table 9. On-farm performance, Stage III (May to June)

	No Soya	Soya
Pens, n.	3	3
Animals, n.	36	35
Weight 4 pen, kg	1,669.3 ± 87.7	1,577.2 ± 161.7
Weight 4 animal, kg	139.1 ± 11.6	135.2 ± 11.0
Daily Weight Index 3 animal, g	5,43.6 ± 125.9 B	673.3 ± 94.8 A
Consumption 3 pen, kg	1,975.7 ± 24.9	1,881.7 ± 83.2
Consumption animal/day, kg	3.7 ±0.1	3.5 ± 0.2
Feed conversion rate 3 pen	7.0 ± 0.9 b	5.4 ± 0.0 a

Different upper case letters indicate differences for p<0.01
Different lower case letters indicate differences for p<0.05

Table 10. Total On-farm performance (February to June)

	No Soya	Soya
Pens, n.	3	3
Animals, n.	36	35
Total Consumption pen, kg	5,313.2 ± 158.5	5,097.7 ± 213.1
Total Consumption animal/day, kg	3.1 ± 0.2	3.0 ± 0.2
Total Daily Weight Index animal, g	698.0 ± 68.7	714.7 ± 66.1
Total Feed conversion rate pen	4.5 ± 0.2	4.4 ± 0.1

Table 11. Slaughtering Performance (carcase weight and yield)

	No Soya	Soya
Pens, n.	3	3
Animals, n.	30	30
Final Live weight, kg	150.6 ± 10.9	147.4 ± 10.3
Hot carcase Weight, kg	122.6 ± 9.9	121.3 ± 8.7
Cold carcase Weight, kg	120.1 ± 9.7	118.9 ± 8.5
Slaughtering hot yield, %	81.7 ± 2.6	82.6 ± 2.1
Slaughtering cold yield, %	80.0 ±2.6	80.9 ± 2.0

Over the second stage, while an average weight difference remained of about 10kg ($p<0.01$), the "soya" group gained in terms of Daily Weight Increase even though worsening with regard to the Feed conversion Index. In the third stage the "soya" group made up all the difference with the "no soya" group, with a Daily Weight Increase ($p<0.01$) and an improved Feed Conversion Index/pen ($p<0.05$). Considering overall values of the whole period of the trial, the two feed trials match each other precisely, with very positive parameters for the type of farming under consideration.

Slaughtering

On slaughtering the two groups were characterised by similar carcase weight and slaughter yield (dead weight/live weight). So far as the cuts were concerned (tables 12 and 13) the "soya" group had increased adiposity with greater bacon weight and yield ($p<0.015$) with reduced loin weight and yield.

The overall results do not show differences between the two groups with respect to total lean and fat cuts (Table 14) or in carcase thickness (Table 15). Nor were differences identified in the meat quality of the different feed groups with the ph measured 45 minutes and 24 hours post mortem, always remaining within the normal range (Table 16).

Table 12. Slaughtering performance: lean cuts

	No Soya	Soya
Pens, n.	3	3
Animals, n.	30	30
Thigh Weight, kg	14.9 ± 1.4	14.9 ± 1.2
Thigh yield, %	24.3 ±1.2	24.5 ± 1.2
Trimmed Thigh Weight, kg	11.1 ±1.1	10.9 ± 0.8
Trimmed thigh yield, %	19.0 ± 1.8	18.8 ± 1.3
Loin Weight, kg	17.2 ± 1.4 a	16.4 ± 1.5 b
Loin yield, %	29.6 ± 2.4 a	28.2 ± 2.6 b
Tenderloin Weight, kg	6.3 ± 0.8	6.2 ± 0.8
Tenderloin yield, %	10.9 ± 1.3	10.6 ± 1.3
Neck Weight, kg	4.1 ± 0.4	3.9 ± 0.5
Neck yield, %	7.0 ± 0.8	6.7 ± 0.8
Boned neck Weight, kg	3.1 ± 0.6	2.9 ± 0.3
Boned neck yield, %	5.3 ±1.0	4.9 ± 0.6
Shoulder Weight, kg	8.9 ± 0.7	8.7 ± 0.6
Shoulder yield, %	15.3 ± 1.2	15.0 ± 1.0

Different lower case letters indicate differences for $p<0.05$

Table 13. Slaughtering performance, fat cuts.

	No Soya	Soya
Bacon Weight, kg	9.4 ± 1.3	9.4 ± 1.6
Bacon yield, %	16.1 ± 2.3	16.1 ± 2.8
Trimmed bacon Weight, kg	3.7 ± 0.6 b	4.3 ± 0.5 a
Trimmed bacon yield, %	6.4 ± 1.0 b	7.4 ± 0.8 a
Cheek Weight, kg	1.3 ±0.2	1.3 ± 0.2
Cheek yield, %	2.3 ±0.4	2.2 ± 0.3
Lard Weight, kg	6.8 ± 0.9	6.3 ± 1.2
Lard yield, %	11.7 ± 1.6	10.9 ± 2.1
Trimmed back lard Weight, kg	2.00 ± 0.5	2.00 ± 0.3
Trimmed back lard yield, %	3.4 ± 0.8	3.4 ± 0.4

Different lower case letters indicate differences for $p<0.05$

Table 14. Slaughtering performance: total cuts

	No Soya	Soya
Pens, n.	3	3
Animals, n.	30	30
Head weight, kg	8.7 ± 0.9	8.5 ± 0.6
Head yield, %	15.0 ±1.6	14.7 ± 1.1
Total lean cuts, kg	44.1 ± 3.4	44.0 ± 2.8
Total Lean cuts yield, kg	73.1 ± 2.1	73.2 ± 2.6
Total fat cuts, kg	16.6 ± 1.9	16.2 ± 2.4
Total fat cuts yield, %	26.9 ± 2.1	26.8 ± 2.6

Table 15. Slaughtering performance: Fat-O-Meater and carcase thickness

	No Soya	Soya
Pens, n.	3	3
Animals, no.	30	30
Hot carcase Weight, kg	122.6 ± 9.9	121.3 ± 8.7
Cold carcase Weight, kg	120.1 ± 9.7	118.9 ± 8.5
SR, mm	35.3 ± 7.6	36.1 ± 7.8
F, mm	56.3 ± 9.2	56.6 ± 6.7
RW	38.5 ± 13.0	34.8 ± 10.4
Lean meat, %	44.9 ± 4.3	44.7 ± 3.6
Average lard at m. GM, cm	4.8 ± 1.2	4.7 ± 0.8
Cranial lard at m. GM, cm	6.0 ± 0.9	5.7 ± 1.1
Caudal lard at m. GM, cm	5.7 ± 1.1	5.5 ± 0.8
Maximum lard at shoulder, cm	6.8 ± 0.6	6.7 ± 0.9
Minimum lard on back, cm	4.0 ± 0.7	4.0 ± 0.6

SR: thickness of back fat (including rind) in millimetres measured at 8 cm off midline of the carcase between 3rd and 4th last ribs
F: Loin thickness between ¾-last rib (mm)
RW: Reflectance

Table 16. Meat quality on slaughter

	No soya	Soya
Animals, n.	20	20
pH 45’ m. Semi-membranous	6.4 ± 0.3	6.3 ± 0.2
pH 45’ m. Longissimus dorsi	6.3 ± 0.4	6.3 ± 0.3
pH 24h m. Semi-membranous	5.9 ± 0.2	5.9 ± 0.2
pH 24h m. Longissimus dorsi	5.8 ± 0.2	5.9 ± 0.3

Live measurements

The last parameter collected was that relating to the animal’s morphometric measurements. As expected the feed group did not generally influence this index (Table 17) but if it was considered by sex (Table 18) the females were smaller both at the withers ($p<0.05$) and at the rump ($p<0.01$) as well as being shorter ($p<0.05$).

Table 17. Morphometric measurements by diet type

	No Soya	Soya
Pens, n.	3	3
Animals, n.	30	30
Height at withers, cm	71.2 ± 4.1	69.4 ± 3.8
Shin circumference, cm	17.3 ± 0.9	17.6 ± 1.0
Thorax width, cm	36.0± 2.0	36.3 ± 2.3
Rump width, cm	32.9 ± 2.0	32.7 ± 1.6
Trunk length, cm	110.3 ± 4.5	110.0 ± 4.2
Thorax circumference, cm	128.3 ± 6.4	129.8 ± 4.4
Height at rump, cm	76.5 ± 4.6	76.0 ± 4.1
Body length, cm	137.4 ± 5.6	137.8 ± 5.2

Table 18. Morphometric measurements by sex

	Male	Female
Pens, n.	3	3
Animals, n.	22	38
Height at withers, cm	71.7 ± 4.2 a	69.5 ± 3.7 b
Shin circumference, cm	17.5 ± 1.2	17.3 ± 0.8
Thorax width, cm	35.7± 2.1	36.4 ± 2.1
Rump width, cm	32.9 ± 1.6	32.7 ± 1.9
Trunk length, cm	111.7 ± 4.0 a	109.2 ± 4.2 b
Thorax circumference, cm	127.5 ± 7.1	129.9 ± 4.1
Height at rump, cm	78.4 ± 4.2 A	75.0 ± 4.0 B
Body length, cm	138.3 ± 5.1	137.1 ± 5.5

Different upper case letters indicate differences for $p<0.01$
Different lower case letters indicate differences for $p<0.05$

Climate data

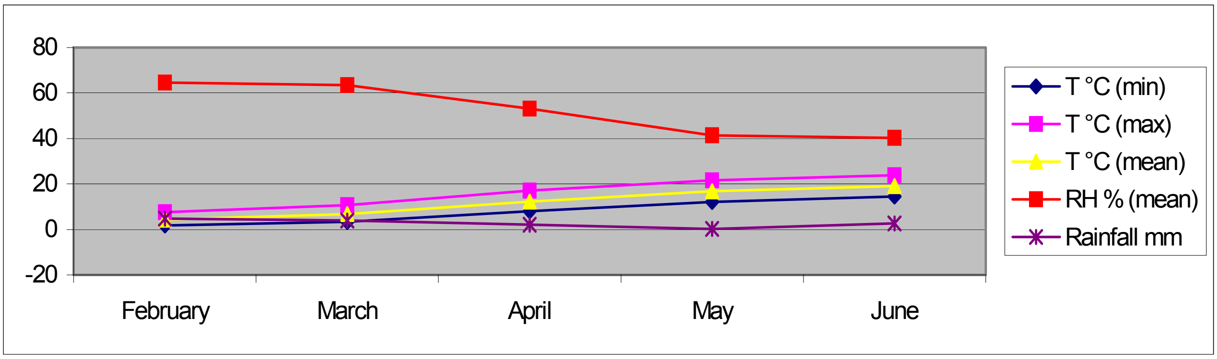
Table 19 and Figure 1 show the average meteorological data collected over the trial. It will be seen that February and March were the months which were the coldest, dampest and with the greatest rainfall.

Table 19. Meteorological Data

	February	*March*	*April*	*May*	*June*
Min Temperature, °C	1.8 ± 3.8 C	3.4 ± 3.5 C	8.0 ± 2.6 B	12.2 ± 4.5 A	14.6 ± 6.7 A
Max Temperature, °C	7.6 ± 3.9 C	10.7 ± 5.3 C	17.1 ± 3.5 B	21.6 ± 5.3 A	23.9 ± 7.2 A
Mean Temperature, °C	4.4 ±3.6 C	6.8 ± 4.2 C	12.3 ±2.9 B	16.8 ± 4.9 A	19.1 ± 7.0 A
Mean Relative Humidity, % Average humidity, %	64.6 ± 17.5 A	63.5 ± 20.8 A	53.1 ± 21.2 AB	41.4 ± 16.8 B	40.3 ± 22.7 B
Rainfall, mm	4.8 ± 8.9 A	3.9 ± 6.3 AB	2.0 ± 3.2 AB	0.2 ± 0.9 B	2.7 ± 5.8 AB

Different upper case letters indicate differences for $p<0.01$

Fig. 1 - Meteorological trend



CONCLUSIONS

The data collected in this experiment do not show particular differences between the two feed groups, confirming the viability of using vegetable protein sources other than soya.

On-farm, after an initial stage when the "no soya" group recorded improved performance, in the second stage the "soya" group recovered ground, being the better of the two in the third stage. In overall results however the two groups are the same. The only quality index differences identified at the point of slaughter were in the loin and bacon of the "soya" group which appeared more adipose.

For meat quality too, no differences in average terms were identified between the two groups.

The trial also highlighted the *Calabrese* pigs ability to adapt well to outdoor production conditions both in production terms and quality terms at the moment of slaughter.

The results are thus of considerable interest for a type of marginal livestock farm, offering a real possibility of farm integration including in consideration of the fact that the use of local raw materials is able to make the resulting product more typical.

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FATTY ACID PROFILE OF INTRAMUSCULAR FAT OF "NERO SICILIANO"FATTENING PIGS FED WITH DIFFERENT DIETS

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SUMMARY - The study evaluated the fatty acid composition of *Longissimus lumborum* (LL) intramuscular fat in "Nero Siciliano" pigs reared on acorn (group A) and barley (group B) during the final stage of fattening. The results of the gas chromatography analysis showed that the LL of group A had a higher content of C18:1ω9 (A 50.47 *vs* B 43.91; $P< 0.001$) and MUFA (A 59.93 *vs* B 48.90; $P< 0.001$) as well as a lower percentage of SFA (A 34.04 *vs* B 39.66; $P < 0.001$) and PUFA (A 6.03 *vs* B 11.44; $P < 0.001$). The atherogenic and thrombogenic indices of group A were also found to be lower: AI (A 0.40 *vs* B 0.44; $P < 0.01$), TI (A 1.01 *vs* B 1.02; $P = 0.212$).

Key words: Nero Siciliano pig, acorn, barley, fatty acid profile.

INTRODUCTION

Both fresh pork and pork products still have a bad reputation due to extensive campaigns that have demonstrated for decades the nutritional deficiencies of pork in terms of its close association with an increased risk of cardiovascular incidents. Even though this was certainly true for the past, today such scruples are completely unfounded from a nutritional point of view. However, the historic prejudice against pork is based on the fact that the consumption of pork has traditionally been associated with cardiovascular risks, given the high cholesterol content as well as the quantity and quality of fat constituents (mainly saturated fatty acids).

In recent years the situation has fundamentally changed in view of the use of new genetic types, an increasingly rational nutrition and the rediscovery of some native Italian porcine breeds that are reared under extensive conditions. Today, pork can be said to contain less cholesterol, and it shows a lower fat infiltration into the muscle tissue. Likewise, the ratio of saturated and unsaturated, monounsaturated and polyunsaturated fatty acids has fundamentally changed.

The aim of this study was to evaluate the fatty acid composition of intramuscular fat in "Nero Siciliano" pigs reared on two different diets during the final stage of fattening.

MATERIAL AND METHODS

The trial was carried out with 24 "Nero Siciliano" pigs. During the final stage of fattening, a group of 12 pigs was fed with acorn (group A) whereas the remaining 12 were fed with barley (group B). At slaughtering, a sample of *Longissimus lumborum* (LL) muscle tissue was taken from each of the 24 carcasses. Each sample was examined for its total fat (AOAC, 2000), followed by an analysis of its acidic composition (Chiofalo et al., 2005). In order to identify the acidic component of each specimen, the fatty acid methyl esters (FAMEs), which had been obtained by direct transesterification of the total fat, with a mixture of 1% sulphuric acid/methanol (ratio 1:9, vol/vol) (Christie, 1993), were prepared.

The methyl esters were then injected into an Agilent 6890 series gascromatograf. The collection and elaboration of the data was supported by software ChemStation. The analyses were performed on a capillary column in a fused silica Omegawax (Supelco) of 30 m x 0.25 mm (length x I.D.) and 0.25 µm (d_f). The cromatographic run was carried out by programming the stove to the following increase in temperature: from an initial isotherm of six minutes at 160°C to 250°C, with a linear increase of 3°C/min. and a final isotherm of 20 minutes. Nitrogen was used as gas-carrier, with a flow of 2 ml/min. The overall duration of each chromatographic run was 56 minutes. All components were identified by comparison with the standard mixtures, and the results were represented on a percent basis, with respect to the totality of identified fatty acids. In addition, the atherogenic index (AI) and the thrombogenic index (TI) were calculated by means of the equations proposed by Ulbricht and Southgate (1991). The obtained data were subjected to the GLM procedure of SAS (2001).

RESULTS AND DISCUSSION

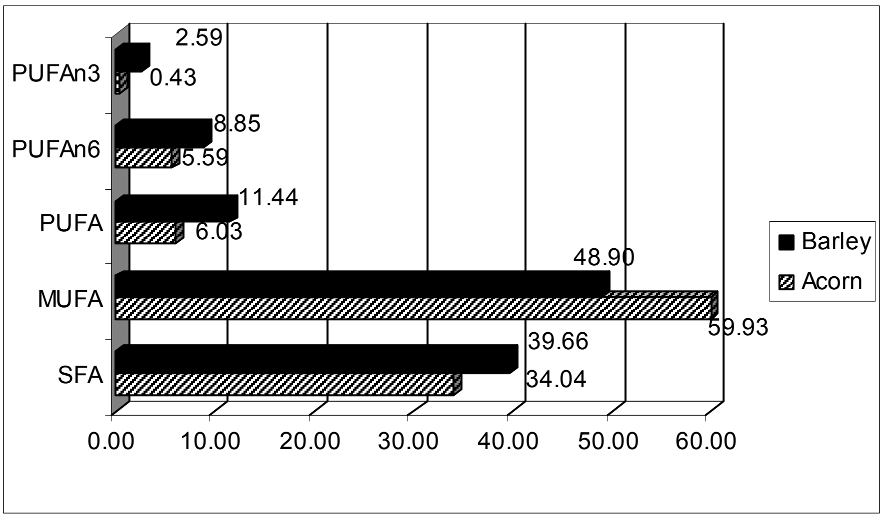
The lipid content of the LL muscle was not influenced by the diet (Table 1).

Table 1 – LL muscle percentage of total fats and several UFAs of nutritional interest

	Acorn	Barley	ES	P
Lipid	3.04	3.68	0.03	0.2400
$C_{18:1\omega9}$	50.47	43.91	0.18	<0.001
$C_{18:2n6}$	4.72	7.10	0.02	<0.001
$C_{18:3n3}$	0.23	0.90	0.05	<0.001
$C_{20:4n6}$	0.87	1.75	0.02	<0.001
$C_{22:5n3}$	0.12	0.85	0.03	<0.001

The gas chromatographic analysis showed that the LL muscle of the pigs that had been fed with acorn (Table 1 and Fig. 1), in comparison with the barley group, manifested a higher and significant percentage of oleic acid C18:1ω9 (A 50.47 *vs* B 43.91; P < 0.001) and total MUFA (A 59.93 *vs* B 48.90; P < 0.001).

Fig. 1. Acidic classes (%) found in the LL muscle



Similar results were found by Daza *et al*. (2007) with regard to the *Longissimus dorsi* (LD) muscle in Iberian barrows of the Torbiscal line that had been fed with acorn and grass during the final 111 days of fattening (C18:1 48.32 and total MUFA 58.12). Tejede *et al*. (2002), analyzing the *Biceps* *femoris* muscle in Iberian and Iberian x Duroc pigs that had been fed on "*Montanera*" (a diet consisting of acorn and pasture), reported the following results: C18:1 (Iberian 54.70 and Iberian x Duroc 56.84) and total MUFA (Iberian 59.96 and Iberian x Duroc 61.67).

Group A, compared to group B, also showed (Fig. 1 and Table 2) a lower percentage of SFA (A 34.04 *vs* B 39.66; P < 0.001) and a better ratio of saturated and unsaturated fatty acids (A 1.94 vs B 1.52; P < 0.001).

Statistically more significant values (Table 1 and Fig. 1) were found for the LL muscle of group B compared with group A for C18:2n6 (A 4.72 *vs* B 7.10; P < 0.001), C18:3n3 (A 0.23 *vs* B 0.90; P < 0.001), total PUFA (A 6.03 *vs* B 11.44; P < 0.001), for the polyunsaturated fatty acids of the series n6 (A 5.59 *vs* B 8.85; P < 0.001) and n3 (A 0.43 *vs* B 2.59; P < 0.001). The higher content of polyunsaturated fatty acids observed in the LL muscle of group B animals might be traceable to their major presence in barley.

As to the dietetic nutritional quality of the LL muscle (Table 2), the atherogenic index (A 0.40 *vs* B 0.44; P < 0.001) and the thrombogenic index (A 1.01 *vs* B 1.02; P = 0.212) resulted favourable to group A. Note that a major content of unsaturated fatty acids in pork has a beneficial effect on human health (Ahn *et al*., 1996).

Table 2 – Ratio of unsaturated and saturated fatty acids and quality indices of the LL muscle

	Acorn	Barley	ES	P
UFA/SFA	1.94	1.52	0.02	<0.001
AI	0.40	0.44	0.01	<0.001
TI	1.01	1.02	0.01	0.212

However, altering the fatty acid profile in pork does not have only positive effects. Increasing the unsaturated fatty acid content of the fat may lead to a softer carcass fat, which might cause problems for the meat processor and retailer (Morgen, 1992). In addition, an increased amount of polyunsaturated fatty acids in muscle renders the tissue more susceptible to lipid oxidation (Allen & Foegeding, 1981). As it has been demonstrated by various authors for different porcine races, we can affirm that the type of diet fed to "Nero Siciliano" pigs during the final stage of fattening does influence the fatty acid composition of the *Longissimus lumborum* muscle.

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PHYSICAL AND CHEMICAL TRAITS OF MEAT OF “NERO SICILIANO” FATTENING PIGS FED WITH DIFFERENT DIETS

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SUMMARY - The study examined the effects of two different feeding strategies used during the fattening period on the physical and chemical meat traits in 24 “Nero Siciliano” pigs. The animals were divided into two groups called Acorn (A) and Barley (B), each consisting of 12 animals, homogenous for live weight, sex, and age. The trial was carried out over a period of 90 days. After slaughtering, M. *Longissimus lumborum* (LL) samples were taken from the left half of each carcass and examined for: pH_1 (45’) and pHu (24h), colour parameters, cooking loss, Warner-Bratzler shear force, and chemical composition. Data were subjected to the GLM procedure of SAS (2001). Comparison of the two groups showed that only a few parameters differed statistically: pH_1, pHu, lightness (L*) and cooking loss.
Key words: Nero Siciliano pig, acorn, barley, meat traits.

INTRODUCTION

The diversification of product quality and production methods, along with food security, the wellbeing of animals, and the conservation of our environment, call for the development of alternative production systems that meet these requirements in a way that is technically, economically, and socially acceptable. “Nero Siciliano” pigs, an autochthonous genetic type that originates from the rural areas of Northern Sicily, are reared in the woods of the Nebrodi mountains. The exceptional market potential of meat and meat products from these animals resides in their high quality food characteristics. Thus, the aim of this study was to assess the dietary-induced effects on the chemical and physical meat traits in the context of different diets used during the final stage of fattening.

MATERIAL AND METHODS

The trial was carried out on 24 “Nero Siciliano” pigs, orchidectomy males, reared in the Nebrodi region of Sicily. The animals were assigned to two groups called Acorn (A) and Barley (B), each group consisting of 12 animals, homogenous for sex and body weight (BW 79.48 ± 0.15 kg). Animals of group A were kept in a wooded area of 12 hectares, appropriately enclosed and fed with acorns during the final stage of fattening (90 days). Animals of group B were reared within an open-air system in the same rural area, and they were fed with germinated barley on a basis of 2.5 kg/pig/d. At the outset, individual microchips (Portorider) were implanted subcutaneously underneath the ear of each animal in order to facilitate an electronic identification at distance. The results of the *in vita* performances are reported in another paper (Zumbo *et al.*, 2007). After 90 days of fattening (in accordance with the experimental design) and a fasting period of 18 hours (ASPA, 1991) the animals were slaughtered. After 24 hours of refrigeration at 4°C, a sample of LL muscle tissue was taken from the left half of each carcass in order to determine the following parameters: (1) water loss: the sample meat slice was placed in a polyethylene bag in moderate vacuum, immersed in a water bath and heated at 75°C. Heating was stopped when the sample reached an internal temperature of 75°C, monitored with a probe (ASPA, 1996). After heating, the sample was cooled under running water for 45 minutes, then blotted and reweighed in order to calculate the cooking-induced water loss. (2) CIE colour (L*, a*, b*) was measured with a spectrometer of an imaging spectral scanner (DV s.r.l. – Italia), using light source D 65; (3) shear force was assessed with a sample of meat that had been cooked in a water bath (1.25 cm diameter removed parallel to the muscle fibre axis and sheared perpendicularly to the same axis), using an INSTRON 5542 equipped with a Warner-Braztler shearing device (speed blade 100 mm/min-1); (4) pH_1 (measured at 45 minutes post-slaughter and pHu (measured at 24 hours post-slaughter after refrigeration at 4°C) were determined with a pH-meter WTN 597-S, equipped with a penetrating probe; and (5) chemical composition (humidity: thermostatic heater ISCO NS9100; ashes: sleeve oven ISCO ISM320; lipids: SOXTEC AVANTI 2050; proteins: Kjeltec 2300 + digestion unit; methods: A.O.A.C, 2005).The found data were subjected to the GLM procedure of SAS (2001).

RESULTS AND DISCUSSION

Measurements of intramuscular pH values offer some useful provisional information about the possible defects of pork meat (PSE, DFD). In this case, the pH_1 (Table 1) resulted lower in those pigs that had been fed with barley (6.12) compared to those fed with acorns (6.28). In both groups, pH_1-values were similar to those found by Zumbo *et al*. (2002) in pigs of the same genetic type, reared under extensive conditions (6.16) and to those found by Pugliese *et al*. (1999) in Cinta Senese (CS) x Large White (LW) pigs that had also been reared in open-air enclosures (6.39), or to those reported by Fortina *et al*. (2001) in Mora Romagnola (MR) x LW pigs (6.40).

Table.1 Physical-chemical traits. Values found in LL muscle samples from groups.

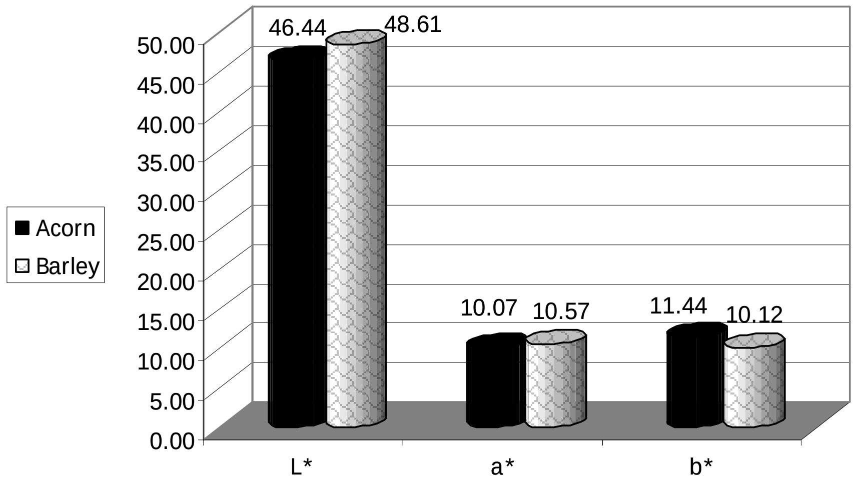
	Acorn	Barley	s.e.	*P*
pH_1	6.28	6.12	0.04	**
pH_U	5.77	5.63	0.03	**
Weight loss (%)	24.60	20.68	0.94	**
WBS (kg f/cm^2)	3.92	3.98	0.16	N.S.
Crude protein (%)	23.22	23.51	0.18	N.S.
Ether extract (%)	3.04	3.68	0.38	N.S.
Ash (%)	1.06	1.09	0.01	N.S.

** P<0.01; N.S. not significant

The pH_1 measured in group A was slightly lower than the one found by Hansen *et al.* (2002) in pigs reared outdoors and fed with acorns (6.28 *vs* 6.41), which might be put down to the different genetic types. The pHu values (Fig.1) resulted higher in group A (5.77) than in group B (5.63). These findings are thus similar to those reported by Corino *et al.* (1996) in pigs fed on a traditional diet (5.70) and to those subsequently found by Simek *et al*. (2002) (5.72). The pHu values measured in both groups were higher than the ones found by Enfalt *et al.* (1997) (5.44 in Yorkshire x Landrace and Duroc x Landrace cross breeds) and the ones reported by Mason *et al.* (2005) in pigs of different genetic types (5.87 in Landrace pigs and 5.84 in Duroc pigs).

Weight loss (Fig.1) in meat samples that had been cooked in a water bath was found to be 24.60% in group A and 20.68% in group B, thus supporting the findings by Zumbo et al. (2002). However, sample weight loss was found to be much lower than the one reported by Pugliese et al. (1999) in LW x CS breeds reared in open-air systems (34.07%). As measured by Warner-Braztler shear force (Fig.1), the meat samples cooked in a water bath showed an almost identical tenderness in both groups (A 3.92 vs B 3.98) that resulted significantly lower than the one found by Pugliese *et al*. (1999) who reported values of 12.17 in LW x CS pigs that had been reared outdoors and of 10.56 in pigs reared indoors (meat samples were also cooked in a water bath). As our findings correspond to those found by Zumbo et al. (2002) for black pigs reared outdoors and fed on a natural diet found within an undergrowth environment (e.g. acorns, tubers, chestnuts, and hazelnuts), it can be hypothesized that these findings are heavily influenced by the respective genetic type. Fig. 1 shows the dietary-induced effects on the colour parameters of the muscle.

Figure 1. Comparison of colorimetric parameters found in LL muscle samples.



Group A showed a lightness (L*) of 46.44, whereas the one found by Hansen et al. (2002) resulted higher (52.8) in pigs that had also been fed with acorns. Compared to Hansen's findings, the index of redness (a*) resulted higher (10.07 vs 8.99), just as the index of yellowness (b*) (11.44 vs 6.04). The colorimetric parameters found for group B were similar to those of group A (L* 48.61; a* 10.57; b* 10.12), and the results of both groups are comparable with the ones reported by Estevez et al. (2002) for three lines of free-range reared Iberian pigs. These findings differ from those reported for the more commonly analyzed "industrial" pigs in a way that leads to the hypothesis that meat traits are strongly influenced by the genetic type rather than by the diet.

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Nero Siciliano pig for the production of "Nebrodi" cured sausage: Effect of some traditional diets on sensorial characteristics

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SUMMARY – The effects of some traditional diets on the sensorial characteristics of "Nebrodi" cured sausages prepared using meat of Nero Siciliano pigs were studied. The animals were fed with germinated barley corn (group A), mash-feed (group B) and germinated barley corn plus citrus pulp (lemon fruits) by-product (group C). The aromatic fraction were analysed by using SPME-GC/MS.
Forty-six volatile compounds (alcohols, ketones, aldehydes, terpenes, oxygenated terpenes, aromatic hydrocarbons and miscellaneous) were identified and quantified in the cured sausages. Higher amount of terpenes was observed in group C (36.44%) compared to group B (28.53%) and A (27.40%); in particular, the most represented components were: limonene (C:11.58%; A:9.11%, B:10.14%), δ-3 carene (C:8.11%; A:5.46%, B:4.97%), β-caryophyllene (C:5.54%; A:4.78%, B:3.83%), myrcene (C:2.44%; A:1.88%, B:1.74%) and β-pinene (C:1.95%; A:1.41%, B:1.44%), well-known for their dietetic and therapeutic properties. Moreover, high amount of aromatic hydrocarbons were observed in the sausages mainly due to estragole (A: 52.76%; B: 44.77%, C: 42.18%).

Key words: Nero Siciliano pig, traditional diets, cured sausage, sensorial characteristics

INTRODUCTION

The preservation of the peculiarity of a typical product requires the identification and quantification of those properties that better describe the characteristics of this product. This can be done by means of microbiological, chemical and sensory determinations (Moretti *et al*., 2004). In particular, the consumer perception of quality of a typical product takes into account the sensorial characteristics which are influenced by different factors such as breed, rearing system and ripening process (Muriel *et al*., 2004).

As regards the typical breeding system of the Nero Siciliano pig, the feeding is usually represented by an extensive management system based on natural pasture and undergrowth products and by a supplementation with grain cereals, legumes and agro-industrial by-product particularly during the period of low pasture availability (Chiofalo *et al*., 2007). In this context the aim of the research was to study the effects of some traditional diets on the aromatic characteristics of "Nebrodi" cured sausages prepared using meat of Nero Siciliano pigs.

MATERIAL AND METHODS

Thirty animals, 16 castrated males and 14 females, were divided into three groups of 10 homogeneous for live weight (32.47±1.74 kg), sex (5 males and 5 females) and age (2 months). The animals were fed with germinated barley corn (group A), mash-feed (group B) and germinated barley corn plus citrus pulp (lemon fruits) by-product (group C). The feeds were analysed (Table 1) using the official methods (AOAC, 2000).

Table 1. Chemical composition of the feeds (percentages on a DM basis)

Feed	Dry matter	Crude Protein	Ether Extract	Crude Fibre	Ash
Germinated barley corn	95.98	12.00	2.46	6.88	2.68
Mash-feed	87.21	14.12	2.29	5.58	2.60
Citrus pulp	94.84	6.25	3.12	18.28	5.02

Animals were slaughtered at 250 days of age and final weight of 70 kg (±5). Concerning the 15 cured sausages, 5 for each group, at 30 days of seasoning and 288g of weight (on average), prepared using the standard technology of the "Consorzio di tutela del suino Nero dei Nebrodi", the aromatic fraction was analysed by means of SPME-GC/MS using the following procedure and expressed as area percentages. Each sample of approximately 3 g were placed in a 10 ml vial and then sealed. The SPME procedure was performed with an AOC-5000 (Shimadzu) autosampler, equipped with a DVB/Car/PDMS 50/30 μm fiber coating (Supelco). Samples underwent an equilibration time of 5 min at 45°C under agitation (500 rpm), whereas the extraction lasted 40 min at the same temperature and agitation. Once extracted, volatile analytes were desorbed into GC injection port at 220°C for 10 min. GC analyses were performed on a GC-2010 (Shimadzu, Milan, Italy), equipped with a FID system (280°C), a split/splitless injector and a GC Solution software for data acquisition (Shimadzu). The oven temperature program was as follow: 40°C (10 min) to 250°C at 7°C/min. All the analyses were carried out on a MDN-5S capillary column, 30 m x 0.25 mm i.d., 0.25 μm d_f. Mass spectrometric information was acquired onto a quadrupole GCMS-QP2010 (Shimadzu), provided with libraries (commercial and home-made). The GC conditions were the same as for GC/FID analyses. The interface temperature was set at 250°C, the ion source at 200°C. Ionization energy: 0.9 kV. Acquisition mass range 40-400; acquisition mode: scan; interval 0.5 sec. Data were subjected to ANOVA (SAS, 2001) considering the variable diet.

RESULTS AND DISCUSSION

Forty-six volatile compounds (alcohols, ketones, aldehydes, terpenes, oxygenated terpenes, aromatic hydrocarbons and miscellaneous) were identified and quantified in the cured sausages.

Table 2. Area percentages of the aromatic compounds identified in the "Nebrodi" sausages in relation to the diet (mean ± SEM)

	Sausage A	Sausage B	Sausage C	SEM
Aromatic hydrocarbon	53.75^{A}	46.52^{aB}	43.20^{bB}	0.9
Terpenes	28.54^{A}	27.40^{A}	36.44^{B}	0.5
Miscellaneous	9.46^{A}	17.47^{B}	9.77^{A}	0.8
Ketones	4.7^{a}	5.39	6.09^{b}	0.3
Alcohols	2.09	1.77^{a}	2.72^{b}	0.02
Aldehydes	1.31	1.18	1.45	0.02
Oxygenated terpenes	0.45	0.46	0.58	0.02

Means on the same column followed by different letters differ significantly (A, B = P<0.01; a, b = P<0.05)

A significant higher amount of terpenes and their oxygenated derivatives, that characterize the volatile fraction of citrus essential oils, was observed in group C compared to group B and A; this could be related to the higher accumulation of the terpenes from the citrus by-products in the adipose tissue of the pigs of group C as reported by Muriel *et al*. (2004). In particular, the most represented components were: limonene, δ-3 carene, β-caryophyllene, myrcene and β-pinene (Table 3) well-known for their therapeutic properties such as: cancer chemopreventive effects, antimicrobial, antifungal, antiviral, antihyperglycemic, anti-inflammatory, and antiparasitic activities (Paduch *et al*., 2007).

Table 3. Area percentages of the most representative terpenes identified in the “Nebrodi” sausages in relation to the diet (mean ± SEM)

Compounds*	Sausage A	Sausage B	Sausage C	SEM	Odour description**
β-Pinene	1.41	1.44	1.95	0.30	*Pine like*
Myrcene	1.88^{a}	1.74^{a}	2.44^{b}	0.50	*Mushroom like fresh*
δ-3-Carene	5.46^{A}	4.70^{A}	8.11^{B}	0.50	-
Limonene	9.11^{a}	10.14^{a}	11.58^{b}	0.30	*Sour lemon like*
β-Caryophyllene	4.78^{a}	3.83^{b}	5.54^{a}	0.50	*Woody, spice, dry*

*Listed according to the elution order.
** From Moretti *et al*., 2004
Means on the same column followed by different letters differ significantly (A, B = P<0.01; a, b = P<0.05)

Moreover, a high amount of aromatic hydrocarbons was observed mainly due to estragole. This flavour component comes from the fennel seeds traditionally added to the raw mixture to make the sausages (Miraldi, 1999); in particular fenchone (A: 2.91%; B: 2.85%, C: 3.24%) , estragole (A: 52.76%; B: 44.77%, C: 42.18%) and (E)-anethole (A: 0.80%; B: 1.75%, C: 0.94%), considered markers of fennel sp., here accounted for about 50% of the identified aromatic fraction, even if they showed quantitative fluctuations in relation to the different amounts of fennel seeds in the original sausages.

Among the groups, no differences were observed for the aldehydes (Table 2), which come from the oxidation of unsaturated fatty acids in meat products (Shahidi and Pegg, 1994); in this context, the low levels of straight chain aldehydes such as hexanal, heptanal and nonanal (Table 4), that account for the “green” aromatic notes (Chiofalo *et al*., 2005), suggest a lipid oxidation (Muriel *et al*., 2004) of low relative importance in the “Nebrodi” cured sausages.

Table 4. Area percentages of the aldehydes identified in the “Nebrodi” sausages in relation to the diet (mean values)

Compounds*	Sausage A	Sausage B	Sausage C	SEM	Odour description**
Hexanal	0.313^{a}	0.310^{a}	0.228^{b}	0.08	*Herbaceous, woody*
Heptanal	0.045	0.046	trace	n.s.e.	*Sour milk, dairy*
2-Heptenal	0.064^{a}	0.143^{b}	trace	n.s.e.	-
1-Octen-3-ol	0.121	0.147	0.098	0.03	-
Phenylacetaldehyde	0.159^{A}	0.051^{B}	0.635^{C}	0.02	-
Nonanal	0.167	0.140	0.208	0.03	*Citrus, malty*
Phenylethyl alcohol	0.083^{A}	0.051^{A}	0.232^{B}	0.02	*Floral notes****
trans-2-Nonenal	0.049	0.067	trace	n.s.e.	*Oats, musty*
Decanal	0.265^{a}	0.182^{b}	0.245^{a}	0.07	-
Trans-2-Decenal	0.044	0.042	trace	n.s.e.	*Orange, fatty fried*

*Listed according to the elution order.
** From Moretti *et al*., 2004;
*** From Chiofalo *et al*., 2005.
Means on the same column followed by different letters differ significantly (A, B, C = P<0.01; a, b = P<0.05)
n.e.s. = no statistical elaboration

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THE EFFECT OF POLYETHYLENE GLYCOL (PEG) ON PROTEIN OUTPUT OF FREE RANGE ALENTEJANO PIGS

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SUMMARY - The effect of PEG treated or untreated acorns fed to Alentejano pigs, on the protein output of the animals were studied. The animals had access to two sown pasture fields based on *Trifolium incarnatum* and *Lolium westerwoldicum* Four dietary treatments were tested with 5 pigs per treatment in a two by two factorial experimental design (factor 1: sown species; factor 2: acorn with or without PEG). Intake of acorns and protein faecal concentration were measured individually. Faecal output was estimated using an external faecal marker (dotriacontane, C_{32}).
Crude protein faecal concentration of animals fed acorns treated with PEG was significantly lower ($P<0,05$) then those fed untreated acorns, suggesting that the PEG may have increased the availability of dietary protein to the animals. However, the estimation of protein faecal output using the n-alkane C_{32} was not significantly affected by the PEG treatment, even though, for the animals in the *Lolium westerwoldicum* pasture, there was a decrease ($P=0,063$) in the total excretion of CP when acorns were treated with PEG.

KEY WORDS: Alentejano pigs; protein output; PEG; acorns

INTRODUCTION

The Alentejano pig, is a breed generally rose under free range conditions and fattened on “Montanheira”, (pasture under evergreen oaks) using the available natural feed resources, mainly acorns and pasture. Acorns have a high content in tannins (Cantos *et. al*, 2003), which are plant polyphenols with the capacity to form strong insoluble complexes with proteins and also other compounds such as starch and cellulose. Therefore, tannins have been reported to reduce protein digestibility and increase faecal nitrogen excretion in mammals (Mangan, 1988). The low performances of Alentejano pigs in “Montanheira”, associated with years of low pasture production, suggest that herbage as a relevant role on the detoxification of tannins from acorns (Almeida, 1986). PEG has a very high affinity to tannins and has been widely used to neutralise the deleterious effects of dietary tannins on nutrient digestibility, particularly protein.

Therefore, an experiment was designed to test the effect of PEG on the protein output of animals fed with acorns and with access to two sown pasture fields (*Trifolium incarnatum* and *Lolium westerwoldicum*). This experiment was part of a larger study, carried out to evaluate the effect of pasture intake on diet utilisation by Alentejano pigs,

MATERIAL AND METHODS

Twenty castrated male Alentejano pigs with an average live weight of 90 kg were allocated to four homogeneous groups balanced by weight and placed in 4 paddocks with access, in each paddock, to a shed with five pens for individual distribution of acorns. Paddock one and two were sown with *Trifolium incarnatum* and paddock three and four with *Lolium westerwoldicum*.

Acorns from *Quercus rotundifolia* were collected from the ground and frozen. Acorns were distributed *ad libitum* to the animals, individually, in two meals per day (8:30 and 15:30) and refusals collected also individually.Animals in paddock one and three received acorns treated with polyethylene glycol (PEG 6000) (12,5g PEG/Kg acorns) and animals in paddock two and four received acorns without treatment. Therefore, four dietary treatments were tested with 5 pigs per treatment in a two by two factorial experimental design. Factor 1: pasture sown species; Factor two:

acorn treatment with or without PEG. The animals stayed in the 4 paddocks, with access to the same diet, for 33 days. During the data collection period, a faecal marker (dotriacontane, C32) spread on 60g doses of ground acorns was distributed individually in the first meal of the day. The data collection period comprised 5 days of adaptation to the faecal marker and 5 days of refusals total collection and faecal samples collection. Refusals were weighed, oven dried, ground (1mm Ø) and pulled per animal. Faecal samples were frozen, freeze dried, ground and also pulled per animal until needed for analysis.

Nitrogen was determined in all samples by the Dumas method in a LECO system (FP528). N-alkanes were extracted and determined by GC-FID as in Dove and Mayes (2006). Tannins in acorns were measured by a protein precipitation method, the radial diffusion assay (Hagerman, 1987). The extraction of tannins from acorn was done with a solution of acetone (70%). Intake of acorns was measured individually and protein faecal concentration was determined. Faecal output was estimated using dotriacontane as an external faecal marker. Diet composition was estimated by the n-alkane technique, using the least-squares diet composition package EatWhat (Dove and Moore, 1996). Estimation of pasture intake was done by the n-alkane technique (Dove and Mayes, 1991), considering the diet composition previously estimated. The data produced was subjected to a factorial ANOVA using the STATISTICA (data analysis software system), version 7 (StatSoft, Inc., 2004).

RESULTS AND DISCUSSION

Dry matter (DM), organic matter (OM) and crude protein (CP) content of the feed resources available for the animals are presented in Table 1. All calculations were done on OM basis, since some animals had high amounts of ashes in the faeces indicating that variable amounts of soil were ingested. Acorns had 40,1g/kgOM of tannins measured as equivalents to tannic acid (radial diffusion assay).

As expected the CP content of acorns was low, what resulted in a low CP intake due to the large percentage of acorns in the diet. Both pastures were in the first stage of development (vegetative state) and therefore with a quite high CP content even on the grass pasture. Pasture 2 had a greater quantity of clover and so its CP content was also the highest.

Table 1: Chemical composition of, acorns and pasture.

	Acorns	Pasture			
		Based on *Trifolium incarnatum*		Based on *Lolium westerwoldicum*	
		1	2	3	4
DM (g/kg)	617,7	299,3	158,5	199,4	204,6
OM (g/kgDM)	980,5	595,5	761,1	743,7	777,3
CP (g/kgOM)	59,2	201,9	269,3	191,0	161,8

Individual intake of acorns (Table 2) was measured directly on the feed troughs and did not differ among experimental groups. Intake of pasture tend to be higher on the *Lolium westerwoldicum* than the *Trifolium incarnatum* based pasture, however this was not statistically significant, probably due to the high standard error observed (0,14). Estimated intake of OM and CP was not different among the four treatment groups.

Crude protein faecal concentration of animals fed acorns treated with PEG was significantly lower ($P<0,05$) then those fed untreated acorns. However, this did not result in a lower total protein excretion when PEG was used, due to an observed increase in the organic matter faecal output and a significant decrease on OM digestibility.

The CP balance (input-output) was negative in all treatments except for animals on *Lolium westerwoldicum* pasture and acorns with PEG. The negative CP balance was probably due to the low CP content of diet (about 74g/kgOM) and also to the formation of insoluble tannin protein complexes. It is interesting to notice that the only treatment where the CP balance was positive is associated with the highest intake of pasture and, therefore, the highest CP intake (Table 2).

Table 2: Intake and output of OM and CP of animals fed acorns treated or untreated with PEG with access to two different pastures

	Pasture							
	Based on *Trifolium incarnatum*		Based on *Lolium westerwoldicum*			Probability of effect		
	A+PEG[1] 1	A-PEG[2] 2	A+PEG 3	A-PEG 4	SE[3]	pasture	PEG	Interaction
Intake of acorns kgOM/d	3,2	2,8	2,8	2,9	0,14	NS	NS	NS
Estimated pasture intake kgOM/d	0,17	0,27	0,65	0,32	0,14	NS	NS	NS
Estimated CP intake g/d	241,8	232,7	265,1	223,9	20,15	NS	NS	NS
Faecal CP gCP/kgOM	224,8	291,3	203,2	304,6	15,65	NS	0,000	NS
Faecal output gOM/d	1236	848	1273	1028	120	NS	0,019	NS
Protein output gCP/d	269,9	241,3	257,6	305,8	19,18	NS	NS	NS
Estimated OM digestibility (%)	63,4	72,2	63,2	67,8	2,99	NS	0,041	NS

[1]A+PEG= acorns treated with PEG; [2]A+PEG= acorns untreated with PEG; [3]SE=Standard error

CONCLUSIONS

PEG treatment did not affect the protein digestibility and reduced OM digestibility. Estimated intake of OM and CP was not different among the four treatments groups although intake of *Lolium westerwoldicum* based pasture tended to be higher. The very low total protein intake was probably responsible for the lack of effect of PEG on the total protein output. The reduction of OM digestibility associated with PEG treatment is difficult to explain and needs further investigation.

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SESSION 4
QUALITY OF MEAT AND MEAT PRODUCTS

NEW METHODS TO ASSESS AND IMPROVE THE QUALITY OF MEDITERRANEAN DRY-CURED HAMS

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SUMMARY - Dry-cured hams are among the most important Mediterranean meat products. In the last decades a number of methods to assess the quality during and at the end of the process have been developed or improved. New research is being done to find genetic markers related with technological quality of raw hams for a correct processing. These genetic markers would be useful in the breeding programs to select animals that “fit” better to the dry-cured ham process, which will improve the homogeneity of dry-cured ham characteristics within batch. There are methodologies based on the detection of biochemical markers such as: proteolytic enzymes related to pastiness in dry-cured ham, peptides which are related to pastiness and bitterness in dry-cured hams, Zn-protoporphyrine IX complex related to colour of hams without nitrate and nitrite and neophytadiene which could be used as a marker of “montanera” feeding system in Iberian pigs. There are also instrumental techniques which could be used to detect “on line” meat with too high/low pH or exudative characteristics by combining pH and impedance probes in the reception step. Several technologies could be useful to determine the fat content: Total Body Electrical Conductivity (TOBEC), Dual Energy X Ray Absorptiometry (DEXA), Ultrasound measurement, low field NMR, Computerised tomography (CT) and NIR. The salting process in dry-cured ham could be improved by using CT and diffusional kinetic models that evaluate the conjoint diffusion of salt and water. The drying process in dry-cured meat products could be improved if the superficial water activity is estimated accurately enough by instrumental techniques (e.g. NIR) and used together with air temperature as inputs of the control system. Moreover, the final product quality could be evaluated by different systems: artificial vision (colour), relaxation tests (texture) and electronic tongue and nose (flavour). All these techniques will be useful, to select and classify the raw materials, to improve the homogeneity of the salting and drying process, to obtain products with a more regular sensory quality and to guarantee the traceability and safety of dry-cured meat products in the different commercialization forms.

Key words: dry-cured ham, quality assessment

INTRODUCTION

Dry-cured hams are among the most important Mediterranean meat products. The criteria traditionally used to classify dry-cured hams were: weight, fat thickness and trimming system. The salting time is fixed for each group according to weight based on the experience of each company. The final product is checked according to texture and sniffed with a fibula to detect off-flavours. In the last decades a number of methods to assess the quality during and at the end of the process have been developed or improved. The aim of this paper is to present and discuss the possibilities of the available methods and other that are under study to assess and improve the quality of dry-cured hams.

METHODS TO ASSESS THE QUALITY OF GREEN HAMS

Technological meat quality

Currently, despite pH is considered a useful parameter to detect hams not appropriate for dry-cured ham manufacture, its use is still reduced. Hams with $pH_{24}>6.20$ are more prone to deterioration and show important appearance, texture and flavour problems (Arnau *et al.*, 1998) and hams with $pH_{24}<5.55$ have a higher incidence of red rings (Arnau *et al.*, 2003) and pastiness (García-Rey *et al.*, 2004; Morales *et al.*, 2007). The pH is determined usually in *Semimembranosus* muscle with electrodes that are adapted to the industrial production lines (Grèbol, 2007). However, the pH measurement does not allow the detection of pale, soft and exudative meat (PSE) in dry-cured ham companies. According to (Oliver *et al.*, 2001) electrical impedance spectroscopy allows to predict a high percentage of PSE hams, and is useful to select hams that could have more pastiness at the end of the process (Guerrero *et al.*, 2004).

Meat composition

Subcutaneous, intermuscular and intramuscular fat contents affect the process and consumer acceptability. Subcutaneous fat thickness is currently evaluated visually, but could be improved with ultrasound sensors and the appropriate software. However, intramuscular fat prediction needs the development of non invasive analysis such as low field X-Ray (Dual Energy X-Ray Absortiometry (DEXA)) and low field Nuclear Magnetic Resonance (NMR). In order to develop and calibrate on-line DEXA and NMR systems, a reliable reference system such as Computed Tomography (CT) could be useful.

CT, common to the medical sciences, has a long and well-documented history as a non-invasive means of studying tissue based on differing X-ray attenuation. In meat science the application of CT has almost exclusively focused on evaluation of porcine gross body composition in vivo and post-mortem (Skjervold *et al.*, 1981; Vangen and Skjervold, 1981; Luiting *et al.*, 1995; Szabo *et al.*, 1999;). This technology presents several drawbacks that prevent its use on industrial scale, cost of equipment and maintenance and safety issues, as CT uses X-rays, which are harmful to human beings. Moreover, this equipment is designed to work in clean environments, as hospitals, and not in meat processing plants.

DEXA allows prediction of fat content by using X-rays of different level of energy. The attenuation of both rays differ depending on the amount of fat, bones,…. By comparing attenuations from both rays, it is possible to obtain a good estimation of fat content. Unlike CT, no special safety measures have to be implemented in facilities where this equipment is used and is much cheaper. However, the information obtained is less accurate than using CT and must be calibrated using other technologies. Some firms have already developed equipments based on DEXA technology for fat content estimation (http://www.productinspection.co.uk/Fat_analysis.htm).

The *NIRS* has been used to determine the feeding system in Iberian pigs as and alternative to gas chromatography and allows the determination of spectra from 120-150 carcasses or animals per hour, which allows the classification in the slaughterhouse before the animals had been dissected (De Pedro *et al.*, 2007).

A prototype imaging transflectance NIR instrument has been developed for analysis of water and fat in fish by Matforsk which could be adapted to fat analysis in external muscles in dry-cured ham.

Raman spectroscopy could also be useful as a non destructive method to determine fatty acid profile measured directly on pork adipose tissue and in melted fat (Olsen *et al.*, 2007).

Electromagnetic scanning for total body electrical conductivity (*TOBEC*) is a rapid and non-invasive method to detect lean content in animal carcasses. The principle is based on the greater electrical conductivity of lean than fat tissue because of a higher content of water and electrolyte present in lean tissue. The difference of electrical conductivity between lean and fat tissues is maximized at low frequencies (Pethig, 1979). It has also been used to predict lean and fat content in order to classify green hams for dry-cured ham production.

As an alternative to physical and chemical procedures, new non-invasive methods such as Pattern Recognition and Image Analysis techniques based on Magnetic Resonance Imaging (MRI) have recently emerged. MRI is a non-invasive method, which uses high magnetic fields to obtain detailed information from any tissue. This technology is widely used in medical applications for patient exploration.

There are several examples of applications of *MRI* in meat science. For instance, (Beavallet and Renou, 1992) analyzed lipid distribution in meat by means of MRI, while (Bonny *et al.*, 2000) characterized muscle structure using such techniques. More recently, (Monziols *et al.*, 2006) quantified muscle, subcutaneous fat, and inter-muscular fat in pork cuts. This technique is also used to show water distribution in relation to drying (Ruiz-Cabrera *et al.*, 2004). In the case of Iberian pork, MRI can help to classify raw loins (Cernadas *et al.*, 2005), can be used to evaluate sensory features and intramuscular fat in dry-cured loin pieces (Antequera *et al.*, 2003) and estimate intramuscular fat levels in *Biceps Femoris* and *Semimembranosus* muscles of Iberian ham (Ávila *et al.*, 2005). The high correlations achieved via Active Contour techniques and MRI, water content and weight, suggest such techniques can be used at the different stages in the ripening process of Iberian hams and that water content and weight loss can be monitored and may be a means for determining the optimal ripening time (Antequera *et al.*, 2007).

However, this technology presents also several drawbacks, equipment and maintenance costs are very high, even for calibration purposes, and safety issues are a serious problem. However, another technology, low field magnetic resonance allows to obtain information on tissue composition, as fat. This technology is much cheaper, but the information obtained is less accurate and must be calibrated using other technologies

Genetic markers.

New research is being done to find genetic markers related with technological quality of raw hams for a correct processing. These genetic markers would be useful in the breeding programs to select animals that "fit" better to the dry-cured ham process, which will improve the homogeneity of dry-cured ham characteristics within batch.

Biochemical markers

There are methodologies based on the detection of biochemical markers such as: proteolytic enzymes related to pastiness in dry-cured ham (Parolari *et al.*, 1994; Toldrá and Flores, 2000) and peptides which are related to pastiness (García–Rey *et al.*, 2004) and bitterness in dry-cured hams (Ruiz *et al.*, 1999).

Differentiation of diet received by animals has been mainly based in the analysis of lipid fraction. Fatty acids of the neutral and polar lipids has been the most used to establish the feeding regime in the final state of fattening (Cava *et al.*, 1997; Coutron-Gambotti *et al.*, 1998; Gandemer *et al.*, 2000; Tejeda *et al.*, 2002). There are a few studies of the unsaponifiable fraction of the intramuscular lipids from Iberian hams (Tejeda *et al.*, 1999; Tejeda *et al.*, 2001a; Tejeda *et al.*, 2001b; Petron *et al.*, 2004), which includes a high number of compounds such as hydrocarbons which could be useful to differentiate between feeding regimes (Tejeda *et al.*, 1999; Tejeda *et al.*, 2001b; Petron *et al.*, 2005). This is the case for neophytadiene, a branched hydrocarbon which is abundant in plant cuticular waxes (Lintas *et al.*, 1979) and was identified only in samples from the pigs fed on *Montanera* (extensive system) and not in the *Pienso* (intensive system) groups (Tejeda *et al.*, 2001b).

METHODS TO IMPROVE THE SALTING AND DRYING TECHNOLOGY

Salt content assessment

The development of improved curing methods for dry-cured meat products is limited by the lack of non-destructive methods for investigating diffusion and the distribution of curing ingredients. Two methodologies previously applied for obtaining non-destructive information about salt distribution in meat are ^{23}Na-magnetic resonance imaging (^{23}Na-MRI) (Guiheneuf *et al.*, 1997; Vestergaard *et al.*, 2004) and CT (Frøystein *et al.*, 1989). While ^{23}Na-MRI is advantageous because it measures directly on one of the nuclei of interest, it is limited by the bore diameter of the magnet, which has to be relatively small (typically 5–10 cm) in order to obtain a sufficiently strong and homogenous magnetic field. In contrast, the gantry opening of a medical CT scanner is approximately 50 cm, allowing for entire cuts to be studied. (Frøystein *et al.*, 1989) demonstrated the applicability of CT for studying salt penetration into dry-cured hams and studied differences in salt penetration in dry-cured ham due to freezing of the raw material. These successful studies have nevertheless not been pursued until recently, perhaps due to the high cost of CT scanners and to insufficient computer processing power for image analysis of the large amounts of data, which CT scanners produce.

CT may prove to be of great value in the near future, both in contexts research and product development as it facilitates the establishment of predictive models which include manufacturing parameters of significance to optimal salt distribution and minimal production time. It could also be a valuable tool for monitoring salt penetration in cured meat and could offer an alternative to labor intensive and destructive chemical analysis, provided that the appropriate calibration curve is developed.

The diffusion coefficient on Na and Cl ions is expected to change as a function of raw material, curing technology and progress of the process. Consequently, the determination of diffusion coefficients for salt diffusing in meat under various conditions relevant to industrial curing is essential, allowing the development of numerical models for salt diffusion in meat. Such models may assist the optimisation of curing processes in industry. A model study on movements of sodium ions in pork loin during brining was carried out by (Vestergaard *et al.*, 2005) using ^{23}Na-magnetic resonance imaging (^{23}Na-MRI). ^{23}Na-MRI profiles suggested that the diffusion of salt into whole meat cuts cannot be described by simple ordinary Fickian diffusion with a constant diffusion coefficient. The diffusion coefficient is suggested to be affected by changes in NaCl concentration, swelling and degree of dehydration (Vestergaard *et al.*, 2005). Thus, NMR offers a unique opportunity to acces non-invasively to the distribution and the state of Na ions in meat tissues.

Drying technology

The drying process in dry-cured meat products could be improved if the superficial water activity is estimated accurately enough by instrumental techniques (e.g. NIR) and combined together with information from other sensors (temperature, humidity rate, air velocity,…). These sensors should be integrated in advanced control architectures and knowledge based systems, so that knowledge from experts could be coded and combined with instrumental techniques. This could lead in the future to autonomous control of dryers and to help to optimize drying process by reducing process time, energy consumption and improve product homogeneity. Recently, water activity was estimated combining readings from temperature, humidity rate and product temperature surface and used to control the drying process of dry-cured meat sausages in a pilot dryer, using an advanced controller as fuzzy control. Results were quite satisfactory (Muñoz *et al.*, 2007), but application of new sensors and advanced control techniques should help in the future to improve the drying process of dry-cured meat products

METHODS TO ASSESS SENSORY QUALITY OF THE PRODUCT AT THE END OF THE PROCESS

The final product colour, texture and flavour could be evaluated by different systems.

Colour assessment

In products prepared using only NaCl, such as Parma ham and some Iberian hams, colour is due to a slow formation of the Zn-protoporphyrine IX complex (ZPP), believed to originate from Mb in which Fe has been substituted by Zn and the heme group separated from the native heme-protein (Wakamatsu *et al.*, 2004a; Møller *et al.*, 2007). This complex is not formed if there is any contact with oxygen or curing substances (Wakamatsu *et al.*, 2004b; Adamsen *et al.*, 2006). More knowledge about these mechanisms of the formation of the stable red pigment in Parma ham would enable the manufacturing of meat products with a desirable and stable red colour without the use of nitrite and nitrate. For this purpose, a light-emitting diode and image analysis was useful to determine the distribution of ZPP in Parma ham (Wakamatsu *et al.*, 2006).

Artificial vision (multivariate imaging)

Artificial vision is widely used in product quality control through colour and some applications have been developed for the food industry. The technology consists in placing an object under uniform lighting conditions and capturing images with a camera, and processing the images to obtain the desired information One of the most promising advantages of this technology is the possibility of obtaining an objective assessment of colour, replacing human subjectivity, and it could allow the automation of colour assessment. However, product heterogeneity and variation in dry cured meat products poses difficulties to the successful development of this technology. Recently artificial vision has been applied to fresh beef colour assessment quite successfully (Tan 2004), although many technical problems remained unsolved. The use of sources with different wavelengths, as UV, could help to improve the accuracy of these systems in food applications..-

Texture assessment

Although the texture profile analysis (TPA) (Bourne, 1978) has been commonly used for evaluation of texture in dry-cured ham, Morales *et al.*, (2007) concluded that the stress relaxation test (SR) at 4ºC evaluates better the softness in BF muscle than the TPA at 4ºC or at 20ºC.

The Near infrared (NIR) Spectroscopy with a fiber optic probe which allows to determine moisture, salt, protein and proteolysis index (Bellatti *et al.*, 2005) has been also used to detect pasty hams (García-Rey *et al.*, 2005).

Flavour assessment

The electronic tongue seems useful to determine NaCl content and water activity (Toldrá *et al.*, 2007) and the electronic nose to determine volatile compounds which are correlated by principal component analysis and neuronal network with several aromatic aspects of Iberian ham (Carrapiso *et al.*, 2001; Santos *et al.*, 2004).

CONCLUSIONS

In the near future, it is expected that different techniques could be available to select and classify the raw materials, to improve the homogeneity of the salting and drying process, to obtain products with a more regular sensory quality and to guarantee the traceability and safety of dry-cured meat products in the different commercialization forms.

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AN ATTEMPT TO PREDICT MEAT DRIP LOSS BY MEANS OF ARTIFICIAL NEURAL NETWORKS

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SUMMARY – The aim of the current study was to evaluate the ability of counter propagation artificial neural networks (CP-ANNs) to predict drip loss using i) a combination of four meat quality traits *pH*, colour parameters *CIE L*, a*, b** (MQ) or ii) visible and near infrared spectra of the meat samples (SPEC). Meat samples (n = 314) were split into training and testing set with regard to the distribution of samples in the *2D* Kohonen map. The CP-ANN models for drip loss prediction were developed on the training set (n = 164, $\bar{x} \pm$ sd = 6.29% ± 3.23%) and tested on the independent testing set (n = 150, $\bar{x}$ ± sd = 6.49% ± 3.22%). For the comparison, the PLS regression model for drip loss prediction was developed. The CP-ANN and PLS models gave similar results for prediction error (RMSE). The $RMSE_{MQ}$ and $RMSE_{SPEC}$ were 2.60 and 2.55% for the prediction of unknown samples with the CP-ANN models and 2.28 and 2.36% for the prediction of unknown samples with the PLS models. The cumulative RMSE for CP-ANN and PLS models were 2.03 and 2.35%, respectively in case of meat quality traits and 2.05 and 2.24%, respectively in case of spectral data. Although the CP-ANN had a comparable ability to predict drip loss using either meat quality traits or spectral information, the latter is of higher practical importance as it has a potential for large scale industrial use.

Key Words: counter propagation artificial neural networks, Kohonen maps (SOM), drip loss, prediction

INTRODUCTION

Drip loss is a technological characteristic of meat that is of great importance for the meat industry and consumers. Although drip loss is rather simple to assess the method is time-consuming, destructive and thus impractical for application under industrial conditions. For this reason drip loss is often indirectly estimated through different simple meat quality measurements like colour and *pH* value or through spectral information. As meat quality measurements or meat spectra explain drip loss only partly the prediction could be improved with the use of suitable statistical tools. Artificial neural networks (ANN) represent modern research tool applicable on many different fields including agriculture and food-processing.

The aim of our study was to apply ANN to predict meat drip loss on the basis of different meat quality traits or on the basis of visible and near infrared spectra and to compare the ability of ANN with the ability of PLS regression for meat drip loss prediction.

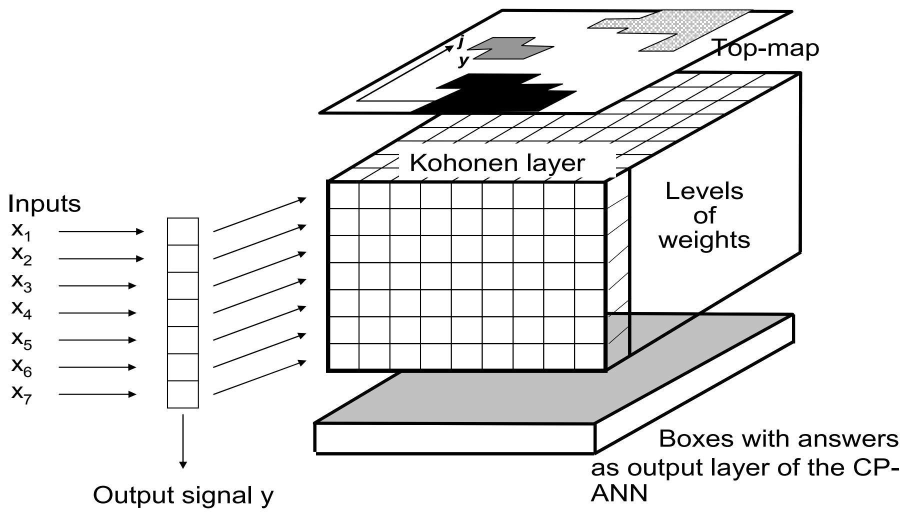
MATERIAL AND METHODS

The Kohonen ANN (*self-organising maps*) belong to the unsupervised strategy of learning, which means that the *2D* mapping is performed regardless of the property or class assigned to individual samples. The main goal of this method is to map objects from *multi*-dimensional into 2-dimensional space on the basis of input data. The counter-propagation ANN (CP-ANN) belong to the supervised strategy of learning and represent an up-grade of unsupervised Kohonen ANN which enables to solve supervised problems (Fig. 1). Beside the input data ($X=(x_1, x_2, ... x_{i1}, … x_m)$) this kind of ANN requires additional layer of neurons called the output layer or the property associated with individual sample

(Y=(y_1, y_2, ... y_{i1}, … y_m)). This (x, y) pairs are the input to the neural network, which is after being trained for certain amounts of epochs, capable of the prediction of unknown samples. Every object/sample excites one single neuron. The algorithm modifies the weight of the neuron with the most intense input or whose weights are the most similar to the input signal and smooths the map by also making modulated changes to neurons in a defined "neighbourhood" of that one. These corrections of weights are made around neuron position in the Kohonen and in the output layer.

In our study the mapping of samples in the Kohonen ANN assisted to split the samples on two adequate sets of objects (training set and test set). The CP-ANN was applied to develop the models for drip loss prediction.

Figure 1. The structure of the CP-ANN



Present study was performed on 314 pig *longissimus dorsi* muscle samples. For all samples colour (*CIE L**, *a**, *b**) and pH_{24} were measured in the abattoir a day after slaughter on the fresh muscle slices. At the same time samples for drip loss and NIR spectroscopy analysis were taken from the same part of loin. Drip loss was determined according to EZ method published by Christensen (2003), where two cylindrical pieces with a diameter of 2.5 cm were weighted, placed in plastic sealable cups, stored at 4°C for 48 hours and then weighed again. Drip loss was expressed as a percentage of initial sample weight. The meat samples were scanned minced 48 hours after slaughter over the wavelength range 400 to 2500 nm (visible and near infrared spectrum) using the laboratory spectrophotometer (NIR Systems model 6500, Silver Springs, MD, USA). Reflectance data were collected as log1/R. The pre-processing of meat quality data (*CIE* L^*, *a**, *b** and *pH*) included normalization: $x_{NEW} = x_{OLD} - \bar{x} / \sigma$. In the case of spectral data instead of the normalisation the raw absorbance spectra (1036 data points) were compressed using the PCA technique to 7 PCA scores.

The meat samples were split on training and test set with regard to the distribution of samples in the *2D* Kohonen map. Splitting was performed separately for the case of meat quality traits and for the case of meat spectra. The training set was used to build the predictive models and the set of independent testing objects for models' validation. Many CP-ANN models for drip loss prediction were developed by varying the net size and number of epochs. Considering the coefficient of determination (R^2) and residual mean squared error (RMSE) for the training and testing the best CP-ANN model was chosen for visual presentation. For the comparison, PLS models were developed and validated on the same training and testing sets of samples. Moreover, the cumulative error ($RMSE_{SUM}$) for both sample sets together was calculated.

RESULTS AND DISCUSSION

In our study the variation range for the drip loss was between 0 and 20 % (Table 1), which is in accordance with the situation in our pig population. The training and testing set were adequate. Varying the net size and the number of epochs 30 different CP-ANN models for drip loss prediction were derived, but the differences between them were small (Table 1).

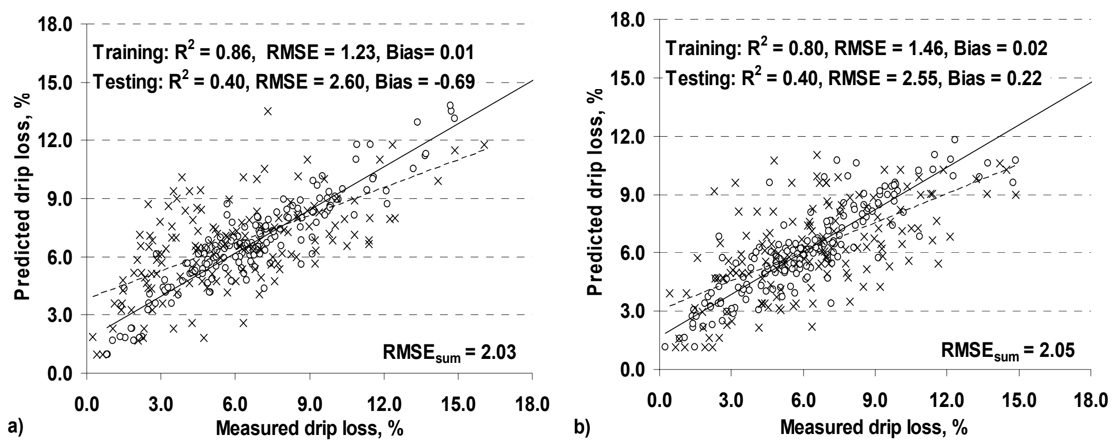
Table 1. Presentation of data sets and prediction of drip loss by different CP-ANN models

	Meat quality traits (L^*, a^*, b^*, pH)		Visible and near infrared spectra (λ = 400 - 2500 nm)	
	Training set	Test set	Training set	Test set
N	164	150	164	150
Variation range, %	6.76 ± 3.10	5.98 ± 3.30	6.29 ± 3.23	6.49 ± 3.22
RMSE, %	0.58 – 1.54	2.68 – 3.08	0.95 – 1.88	2.55 – 2.99
R^2	0.75 – 0.97	0.25 – 0.40	0.66 – 0.95	0.21 – 0.40

R^2 – coefficient of determination
RMSE – root mean squared error

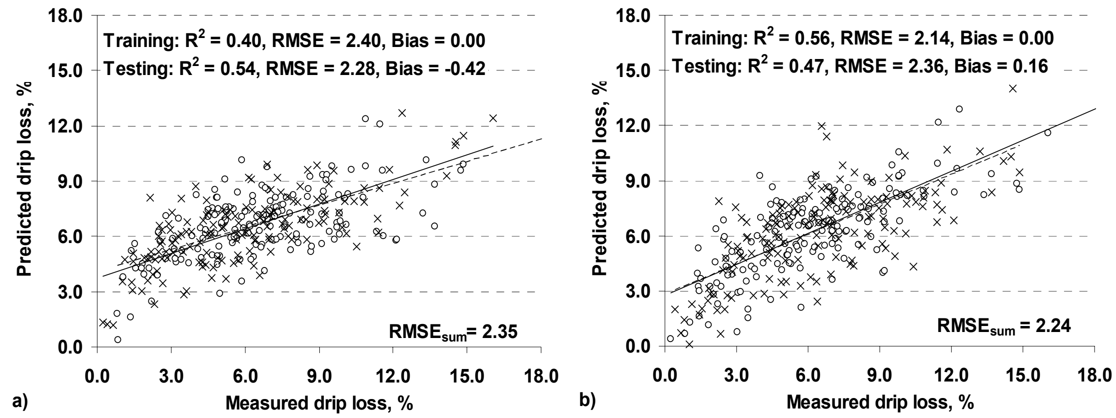
For prediction of drip loss on the basis of meat quality traits the model with 14×14 neurons and 100 epochs showed the best performance. On the basis of spectral information the best model had 13×13 neurons and 150 epochs. The prediction of drip loss with the selected CP-ANN models is represented in figure 2. For the comparison, the prediction of drip loss using PLS regression models is represented in figure 3.

Figure 2. Prediction of drip loss (%) a) on the basis of *pH*, *CIE L**, *a** and *b** by the best CP-ANN model (14 × 14, 100 epochs) and b) on the basis of spectral information by the best CP-ANN model (13 × 13, 150 epochs)



•, ——— testing on training set of samples; ×, – – – testing on an independent set of samples

Figure 3. Prediction of drip loss (%) a) on the basis of *pH*, *CIE L**, *a** and *b** by the PLS regression model and b) on the basis of spectral information by the PLS regression model



•, ——— testing on training set of samples; ×, − − − testing on an independent set of samples

The statistical parameters show comparable ability to predict drip loss of unknown samples using CP-ANN or PLS regression. The $RMSE_{MQ}$ and $RMSE_{SPEC}$ were 2.60 and 2.55 % for the prediction of independent samples with the CP-ANN models and 2.28 and 2.36 % for the prediction of independent samples with the PLS models. As a consequence of better training results (lower RMSE on training set) for the CP-ANN models the cumulative RMSE in prediction by CP-ANN is about 0.3 % lower in comparison to the cumulative RMSE of PLS models. This could be explained by the fact that CP-ANN models belong to the so called data driven models, where the data form the type of model/function and the size of coefficients, in contrast to regression where the type of function/model is predefined. The cumulative RMSE for CP-ANN models were 2.03 % and 2.05 % in case of meat quality traits and in case of spectral data, respectively. In view of the variation range for the drip loss (0-20 %) the error of 2% might seem large. Therefore, the error of prediction must be discussed with regards to the reproducibility of the reference method, which is 1.3 % for the case of drip loss (data not shown). Considering this, the error of prediction by CP-ANN is relatively good. Although the CP-ANN showed comparable ability to predict drip loss using either meat quality traits or spectral information, the latter is of higher practical importance as it has a potential for large scale industrial use.

CONCLUSIONS

In the current study the CP-ANN were found to have satisfactory ability to predict drip loss on the basis of meat quality traits or on the basis of spectral information. The prediction ability is comparable, but of higher practical significance when using meat spectra as it has a potential for application under industrial conditions. Despite relatively good ability of CP-ANN to predict meat drip loss a suggestion to achieve better results would be to adopt more accurate or repeatable reference method and/or to try with a different chemometric approach (back propagation ANN or genetic algorithm, etc.).

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MICROBIAL SURFACE COLONIZATION IN NEBRODI *SALAME*

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SUMMARY - Within the framework of a three-year project, identification of spontaneous fungal species grown on the surface of *salame* produced in different ripening environments has been carried out during the first year. Isolates that proved safe on the basis of their biochemical characteristics and that were appreciated for their ability to give *salame* satisfactory appearance as well as good technological and sensory traits have been investigated for possible use as starter. In a first step, both quantitative and qualitative analyses were carried out on salami casings produced in the Nebrodi area or processed at SSICA pilot plants. In the following phase of the project, the biochemical characteristics of the strains were compared by lipolysis and preoteolysis tests at different temperatures. The results obtained showed that both enzymatic activities increased with incubation time and temperature. In particular, morphologically similar species belonging to *Penicillium* subgenus *Penicillium* proved to have considerable lipolytic activity and a higher variability at proteolytic activity.

Key words: *Salame*, moulds, lpiolysis, proteolysis.

INTRODUCTION

The surface of ripened meats tends to be quickly colonized by mycetes, such as moulds and yeasts (Samelis and Sofos, 2003). In general, at the beginning of the ripening process yeasts are the predominant on the surface; at the end of the ripening process, mycoflora is evidently represented by moulds, while yeasts may vary from one to two logarithmic cycles.

The prevalence of moulds is essentially due to: (i) the progressive reduction of surface water activity in sausages; (ii) the invasive way moulds grow on the surfaces; (iii) the substantial production of conidia; and (iv) the production of secondary metabolites, that can inhibit yeasts and other micro-organisms.

In general, surface moulding is considered peculiar in order to obtain high-quality traditional products, since it has been demonstrated that the mycelium: (i) prevents excessive drying; (ii) protects fat portions from oxidation because it metabolizes and consumes peroxides; (iii) reduces the O_2 levels on the surface of the product, thus avoiding oxidative processes and improving meat colour; (iv) contributes to enhancing the flavour of the final product, because it breaks up fats, proteins and lactic acid, thus favouring pH increase; and (v) makes sausage peeling easier thanks to the differentiation of the fungal basal hyphae into a sort of root called "rhizoid", which can penetrate the casing (Grazia et al., 1986).

As regards salami ripened in traditional and uncontrolled environments, their moulding is essentially due to the natural selection of those autochthonous strains which better adapt themselves to the thermohygrometric conditions applied. The above strains mainly belong to *Penicillium* subgenus *Penicillium* that may overcome those belonging to more xerophilic genera, such as *Aspergillus* and *Eurotium* (Spotti et al., 2007).

MATERIAL AND METHODS

<u>Moulds and yeasts counts and identifications</u>

The casings of Nebrodi *salame* were tested at different ripening times. They were weighed, placed in Stomacher bags with sterile water containing 0.1% Tween 80, and blended by using a

Stomacher for 5 minutes. Then, moulds were counted as cfu/cm^2 of casing. Each of the strains isolated was identified according to techniques proposed by Pitt e Hocking (1997) and by Samson (2004).

Lipolytic and proteolytic activities

Enzymatic tests were carried out according to techniques proposed by Paterson and Bridge (1993). Each fungal strain was inoculated into a test tube containing solid medium and incubated at 10, 14, and 18°C for 7-21 days. For each strain, analyses were done in duplicate.

RESULTS AND DISCUSSION

Moulds and yeasts counts and identifications

In the first phase of the project, both quantitative and qualitative analyses were carried out on casings of *salami* produced both in the Nebrodi area and at the SSICA pilot plants, using the same raw meat and different technologies (Tables 1 and 2). Besides, at the SSICA the results concerning salami obtained with meat from “Nebrodi black pig” were compared with those concerning *salami* produced with meat from “hybrid pig” (Table 2 and 3).

Samples were tested at different ripening times (from 28 to 60 days as regards salami from the Nebrodi area; from 7 to 75 days as regards salami produced at the SSICA by using either ”Nebrodi black pig” or “hybrid pig” meats). For each fungal species, the lower and the higher values of colonies forming unit (cfu) detected on the total amount of samples have been reported.

Table 1. Moulds and yeasts counts on casings of different *salami* produced in the Nebrodi Area by using “Nebrodi black pig” meat, at different ripening times.

Fungal species	Salami from “Nebrodi black pig”			
	28 days		60 days	
	lower cfu/cm^2	higher cfu/cm^2	lower cfu/cm^2	higher cfu/cm^2
P. gladioli	$2,1 \times 10^4$	$1,3 \times 10^7$	$2,7 \times 10^6$	$2,1 \times 10^7$
P. aurantiogriseum	$1,4 \times 10^4$	$7,0 \times 10^6$	$1,1 \times 10^5$	$7,7 \times 10^6$
P. brevicompactum	$7,0 \times 10^4$	$1,4 \times 10^6$	$1,3 \times 10^6$	$4,6 \times 10^6$
P. nalgiovense	$2,3 \times 10^3$	$1,4 \times 10^6$	$< 10^4$	$1,8 \times 10^7$
P. griseofulvum	$3,0 \times 10^4$	$2,1 \times 10^6$	$1,1 \times 10^6$	$1,3 \times 10^7$
Eurotium spp.	$5,8 \times 10^4$	$2,7 \times 10^5$	$< 10^4$	$8,1 \times 10^4$
P. nordicum	$2,8 \times 10^5$	$1,2 \times 10^6$	$< 10^4$	$< 10^4$
yeasts	$2,6 \times 10^5$	$2,4 \times 10^7$	$3,8 \times 10^6$	$1,1 \times 10^7$

Table 2. Moulds and yeasts counts on casings of *salami* produced at the SSICA pilot plants by using “Nebrodi black pig” at different ripening times.

Fungal species	Salami from “Nebrodi black pig” (cfu/cm^2)		
	7 days	28 days	75 days
P. gladioli	$< 10^2$	$1,7 \times 10^4$	$6,0 \times 10^5$
P. solitum	$< 10^2$	$2,6 \times 10^4$	$2,0 \times 10^4$
P. aurantiogriseum	$1,6 \times 10^4$	$2,6 \times 10^4$	$< 10^4$
P. brevicompactum	$< 10^2$	$1,7 \times 10^4$	$1,0 \times 10^5$
P. nalgiovense	$< 10^2$	$5,1 \times 10^4$	$< 10^4$
yeasts	$5,5 \times 10^6$	$1,7 \times 10^6$	$3,8 \times 10^7$

Table 3. Moulds and yeasts counts on casings of *salami* produced at the SSICA pilot plants by using "hybrid pig" meat, at different ripening times.

Fungal species	Salami from "hybrid pig" (cfu/cm^2)		
	7 days	28 days	75 days
P. gladioli	$2,1 \times 10^{3}$	$6,2 \times 10^{3}$	$6,0 \times 10^{5}$
P. solitum	$3,1 \times 10^{2}$	$< 10^{4}$	$4,0 \times 10^{5}$
P. aurantiogriseum	$< 10^{2}$	$1,5 \times 10^{4}$	$< 10^{4}$
P. brevicompactum	$< 10^{2}$	$3,1 \times 10^{4}$	$< 10^{4}$
P. nalgiovense	$< 10^{2}$	$1,5 \times 10^{4}$	$6,0 \times 10^{4}$
yeasts	$1,4 \times 10^{5}$	$7,7 \times 10^{6}$	$1,4 \times 10^{7}$

The analyses of the casings allowed to monitor fungal growth as a function of RH and T conditions applied during ripening and to evaluate any possible interaction between the species isolated (*P. gladioli, P. aurantiogriseum*, *P. solitum, P. brevicompactum*, *P. nordicum*, *P. griseofulvum,* and *P. nalgiovense*). These ones proved to overgo 2-3 logarithmic increments and so did yeasts, which overwent 2 logarithmic increments during the ripening process.

In particular, among the species isolated *P. solitum* and *P. nordicum* have been detected. Their presence at high concentrations must be always kept under control, since they are considered undesiderable moulds because of the heavy conidiation (the former) or the ability to produce secondary toxic metabolites (the latter).

Lipolytic and proteolytic activities

In the following phase of the project, the biochemical characteristics of the isolated strains were studied by lipolysis and proteolysis *in vitro* tests at 10°C, 14°C and 18°C. Their enzymatic activities have been then paired with those of moulds isolated on salami produced in the Northern Italy, in order to evaluate the influence of environmental factors on fungal metabolism and the possible adaptation of moulds tested to the nutritional niches available in a specific area.

The results of in vitro enzymatic tests show that both enzymatic activities increased with incubation time and temperature (Figs 1 and 2). In general, at 10°C the enzymatic activities of the isolates tested proved to be very reduced than those registered at 14°C (+ 9-17,5% as regards lipolysis; + 6-21% as regards proteolysis at 14°C) and at 18°C (+ 19-25% as regards lipolysis; + 3-27% as regards proteolysis at 18°C).

In particular, morphologically similar species belonging to *Penicillium* subgenus *Penicillium*, such *as P. aurantiogriseum*, *P. solitum, P. brevicompactum*, *P. nordicum*, and *P. griseofulvum*, proved to have considerable lipolytic activity, that is important since it is responsible for formation of aroma compounds typical of salami. On the contrary, the above species showed greater differences in their proteolytic activity which resulted lower than the lipolytic one, but which is anyway less significant, since it is mainly carried out by fermentative bacteria grown in the mince and by proteases native to meat.

Figure 1. Lipolytic activity of the strain from the Nebrodi area and from Northern Italy, tested at different temperatures and times of incubation

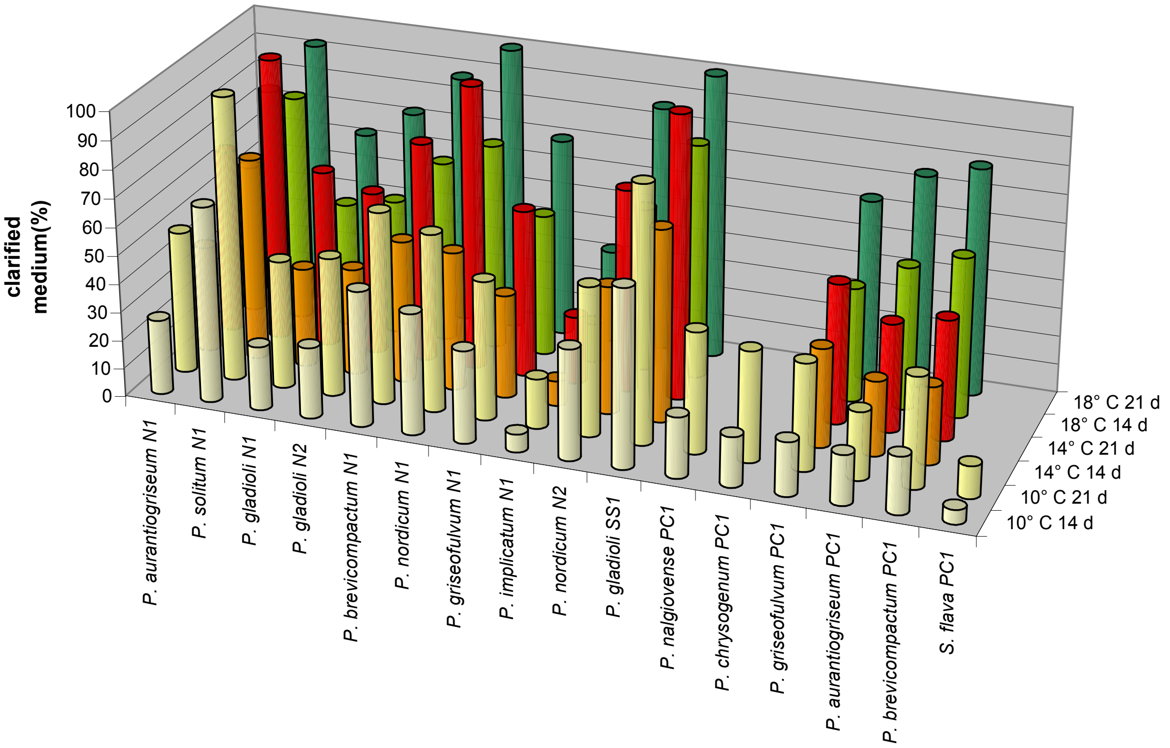
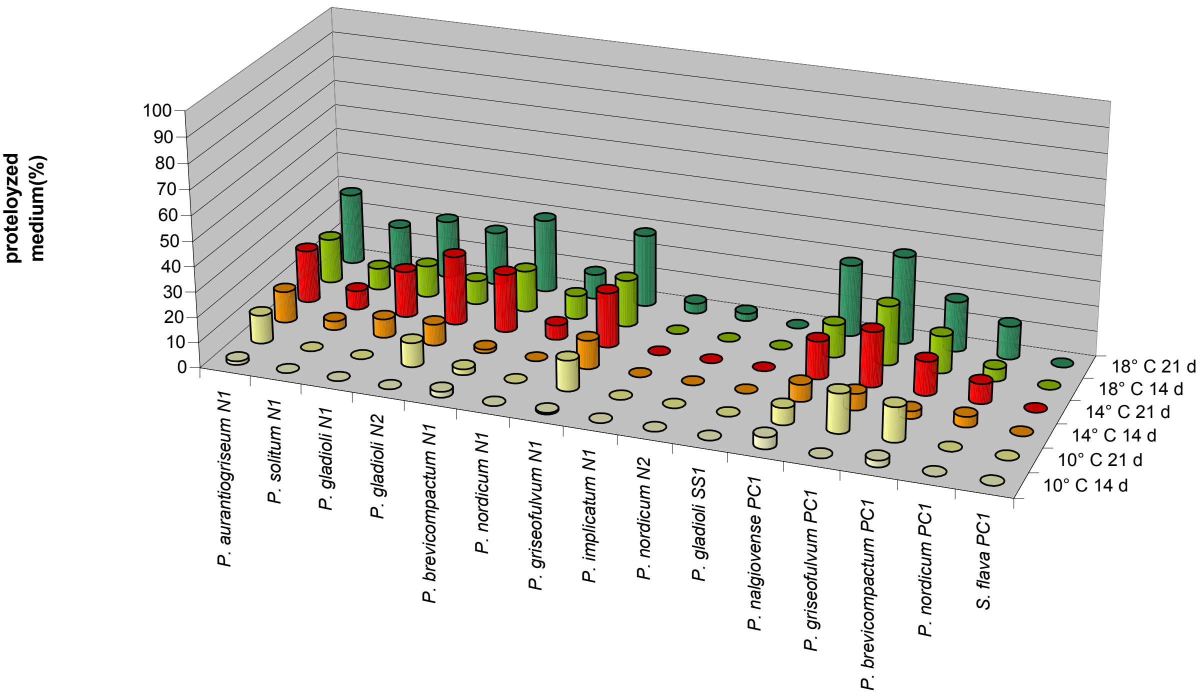


Figure 2. Proteolytic activity of the strain from the Nebrodi area and from Northern Italy, tested at different temperatures and times of incubation



CONCLUSIONS

It will be taken into account the possibility of selecting and cultivating those isolates producing greyish or whitish conidia, dominating over undesiderable fungal strains, and proved safe on the basis of their biochemical characteristics, for possible use in the further stages of the project as starter cultures to be inoculated on salami casings.

Besides, it will be studied the possibility of using fingerprinting techniques for the characterization of autochthonous fungal isolates, as it already happens for bacteria added to minced meat before its casing, in order to give a further tool to assure both traceability and tipicity of regional products.

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CARCASS AND MEAT QUALITY OF PORC NEGRE MALLORQUÍ (MAJORCAN BLACK PIG)

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SUMMARY - Sixty-six heavy pigs of the Majorcan Black Pig breed were reared in a typical production system on Mallorca, and slaughtered in commercial conditions at an average age of fourteen months (live weight of 158.4 ± 18.7 kg). Their carcass and meat quality were evaluated. The killing out percentage was 80.4 ± 0.85 % and the flare fat weighed 6.0 ± 1.4 kg. Back fat depth measured at the last rib in the midline was very high (72.1 ± 12.3 mm) in comparison with other breeds including the Iberian breed. A big variation was observed for both pH45 and pH24 determined respectively in the m. *Semimembranosus* and in the m. *Longissimus lumborum*. These results indicate genetic differences within the breed as well as the influence of the *ante mortem* treatment. Majorcan Black Pigs showed high levels of intramuscular fat content (IMF) (8.88 ± 2.83 %) and pigment content (1.97 ± 0.40 mg myoglobin/g of muscle) in relation to the Iberian breed. The L* value of colour (CIELAB space) of the loin was affected by IMF (R^2 = 0.24) and pH24 (R^2 = 0.33), and the a* value by pigment content (R^2 = 0.29). Back fat depths and IMF were not affected by carcass weight. No gender influence was observed for any of the studied variables.

Key Words: carcass, meat quality, Majorcan Black Pig

INTRODUCTION

Majorcan Black Pig is a typical breed from Mallorca, situated in the Mediterranean, off the coast of Catalonia, in Spain, that has been recognised as an autochthonous and endangered extensive pig breed by the Spanish Ministry of Agriculture, Fish and Food. Its genealogic origin is under research. During the history of the Island there were some factors that affected the breed's survival, such as the Arabian domination, diseases such as the African swine fever, and recently, the introduction of other intensive production system breeds favouring leaner carcasses, such as the Majorcan Black Pig line. The Majorcan Black Pig exploitations were reduced to family farms level, but thanks to the Administration and the Majorcan Black Pig Producers Association's efforts, and the breed herd book appearance in 1997, at present there are about 1300 sows and 120 boars. This recovery is also due to the Protected Geographical Indication (PGI) qualification of its best known product in 1994: the *Sobrassada de Mallorca de Porc Negre* , which gives an added value to this meat product and is an economical incentive for the local meat industry.

The aim of this preliminary study was to analyze the carcass and meat quality characteristics of the Majorcan Black Pig breed in their typical production system.

MATERIAL AND METHODS

In this study, 66 pigs (27 castrated males and 39 gilts) of Majorcan Black Pig breed, proceeding from several Majorcan farms, were slaughtered in three batches at the same abattoir, at an average age of 14 months (live weight 158,4 ±18,7 kg). Three slaughterings took place, two in autumn and one in spring. The animals were reared in extensive conditions, with a feeding regime based on pasture, cereals (barley and rye), legume seeds, figs, almonds, acorns and several Mediterranean shrubs. Once fasted, they were transported to the slaughterhouse between three and five hours before the slaughter. The stunning method used was the electrical stunning (250 v. and 50 Hz.), lasting about four seconds per animal. Animals were eviscerated and split in two halves using an electrical saw. At this point the flare fat was removed and weighed on an industrial scale. The cutting of the carcasses was carried out between approximately 8-12 hours *post-mortem*.

The carcass quality parameters were evaluated on the left half of the carcass. Carcass length was measured from the first rib to the beginning of the *simfisis pubica,* and the loin length from the *atlas* to the first lumbar vertebra, using a tape measure; minimum back fat thickness over the *gluteus medius* muscle (MLOIN), back fat thickness at the last rib level (Back fat), and back fat thickness at the first rib level (Esp1C), were measured in the mid-line using a ruler.

Muscle pH was measured using a Knick pH meter (Knick, Berlin, Germany) with a xerolyt probe, at 45 minutes *post-mortem* in the *Longissimus thoracis* (LT) muscle at the last rib level (pH45LT), in the *Semimembranosus* (SM) muscle (pH45SM), and at 24 h *post-mortem* (ultimate pH) in a sample of *Longissimus lumborum* (LL) muscle*,* at the first lumbar vertebra level (pH24LL). All measurements carried out at 24 hours were taken at the first lumbar vertebra level. Electrical conductivity was measured in the LL at 24 hours *post-mortem* using a Pork Quality Meter (PQM-I/KOMBI, Intek Klassifizierungs-technik, Aichach, Germany) (ECuLL).

The subjective evaluation of LL colour and marbling were performed by two experienced observers following the Japanese colour scale as reference (1: very pale, 6: very dark), (Nakai, 1975), and the National Pork Producers Council (NPPC) pattern (NPPC, 1999) respectively. The objective measurement of LL colour was carried out using a Minolta CR-200 chromameter, (CIE L* a* b* (CIE, 1976). The LL muscle intramuscular fat content was measured using the Near Infrared Transmittance technology (NIT) following the standard procedure described by Olivan et al. (2000) and Gispert *et al*. (1997). The determination of the pigment concentration in LL was done following the method of Trout (1991).

RESULTS

Carcass quality

There is no referenced information about carcass and meat quality values in Majorcan Black Pig breed, so the obtained results cannot be compared with former experimental data from this breed. The data from males and gilts were analyzed separately and no significant differences were found between sexes in most of the carcass and meat quality variables. Due to the heterogeneity of the farms of the animals studied, the batch effect and the feeding regime and energy requirements depending on season they are slaughtered, the animals' live weight presents a high standard deviation (S.D.).

Mean, S.D., minimum and maximum values for carcass quality parameters are shown in Table 1. The reported live weights in the present trial went from 124.0 to 187.0 kg, considered as normal values for this heavy weight breed. The carcass weight mean was 117.1 ± 16.45 kg, and the killing out percentage was 80.4 ± 0.85%. This high value is because heavier animals present higher killing out rates compared with commercial ones. Flare fat weight was higher (6.0 ± 1.43 kg) than other commercial breeds in intensive conditions. Majorcan Black Pig flare fat is appreciated by local meat industry, and it is used mainly in the *sobrassada* elaboration process, therefore is an important attribute to bear in mind in future selection programs. Carcass length was similar to other heavy weight breeds as the Iberian pig slaughtered at the same weight (82.6 cm) (Benito *et al*., 2000). Regarding fat thickness high values were found, 65.7 ± 10.5 mm in MLOIN, 72.1 ± 12.4 mm in back fat, and 89.3 ± 13.3 mm in Esp1C. Animals of 130 kg of carcass weight from Iberian pig breed showed values in back fat level about 60 mm, less than those observed in Majorcan Black Pig in the present study (Ramírez and Cava, 2007; Daza *et al.*, 2005). The obtained fat thickness values in the studied breed are much more higher than the observed ones in commercial breeds (Gispert *et al*., 2007).

Table 1. Mean, S.D., minimum and maximum values of carcass quality variables in the Majorcan Black Pig breed.

Variable	Mean	S.D.	Min.	Max.
Live weight (kg)	158.4	18.73	124.0	187.0
Carcass w. (kg)	117.1	16.45	87.4	151.5
Killing out (%)	80.4	0.85	78.3	81.4
Flare fat (kg)	6.0	1.43	4.2	10.4
C. length (cm)	82.6	6.06	69.0	92.5
MLOIN (mm)	65.7	10.53	40.0	92.0
Back fat (mm)	72.1	12.35	50.0	110.0
Esp1C (mm)	89.3	13.27	70.0	140.0

MLOIN: minimum fat thickness over the *gluteus medius* muscle; Back fat: fat thickness at the last rib level; Esp1C: fat thickness at the first rib level.

Meat quality

All the meat from the Majorcan Black Pig is used in the *sobrassada* elaboration, so there is no specially appreciated group of muscles in preference to another one. The main criterion for choosing the loin (*Longissimus dorsi)* to measure the meat quality parameters were the numerous bibliographic references that exist to this muscle in other breeds. However, it would be interesting to know the technological characteristics of the LL muscle for the next proposals, as the development of new products as *carpaccio* and some other cured products. Mean, S.D., minimum and maximum values for meat quality parameters are shown in Table 2..

The pH values measured at 45 minutes *post-mortem* in LT and SM muscles (6.19 ± 0.282 and 6.25 ± 0.282 respectively), and measured at 24 *post-mortem* in LL muscle (5.87 ± 0.330) are at the normal pH benchmark. Regarding minimum values of pH45 in both muscles, some cases of exudative meat, and consequently a low water holding capacity, could be observed. These results could indicate that a high variation in the biochemical characteristics of the LL and SM muscles exist in this breed. On the other hand, values of pH24 were observed higher than 6.0, that indicate that some animals' muscles developed DFD characteristics, or that they perhaps had a higher percentage of oxidative fibres. These results are in accord with the L* values obtained, some of them very low and typical of dark coloured meat. Subjective evaluation of the meat colour supports these objective values obtained by the Minolta chromameter. The animals studied presented an average electrical conductivity of 2.51 ± 0.822 mS, so the water holding capacity of their meat would be classified as acceptable. Objective colour was defined by three components: lightness (L*), redness (a*) and yellowness (b*). Meat from the breed studied can be considered redder and darker than commercial intensive breeds, as shown by the L* and a* values (44.13 ± 3.128 and 9.83 ± 1.639 respectively). These values were similar to those obtained in Iberian pig. The high values of a* might be explained because this colour component is related to the muscle pigment content, which increases during the life time of the animals, therefore animals sacrificed at heavy weights are more prone to produce more intense red meat (Garcia-Macias *et al*., 1996). Accordingly, the pigment content of the muscle was very high, even higher than the values obtained in other studies with Iberian pigs (Ramírez and Cava, 2007; Serra et al., 1998) which are also slaughtered at high weights. Subjective colour comprised a range of values from 2.0 to 4.5. The loin marbling was subjectively appreciated and gives an idea of the amount of intramuscular fat (IMF) contained in the muscle. In this case marbling scores were high (2.8 ± 0.89), indicating high levels of IMF. The analysis, by means of the objective and the rapid NIT (Near Infrared Transmittance) technique for the determination of IMF content, gave mean values of 8.88 ± 2.832%, with a maximum of 17.30%. The obtained results of IMF in Majorcan Black Pig are quite higher than the ones observed in the Iberian breed, which IMF levels do not usually achieve levels higher than 7% (Ramírez and Cava, 2007; Benito *et al*., 2000).

Table 2. Mean, S.D., minimum and maximum values of meat quality variables in the Majorcan Black Pig breed.

Variable	Mean	S. D.	Min.	Max.
pH45LT	6.19	0.282	5.40	6.78
pH45SM	6.25	0.282	5.61	6.87
pH24LL	5.87	0.330	5.44	6.63
ECuLL (mS)	2.51	0.822	0.70	4.20
L*	44.13	3.128	36.05	53.60
a*	9.83	1.639	6.05	13.61
b*	1.42	2.329	-3.08	5.67
JCS	3.4	0.57	2.0	4.5
IMF (%)	8.88	2.832	4.44	17.30
Marbling (NPPC)	2.8	0.89	1.0	5.0
Pigments	1.97	0.404	1.47	2.93

LT: *Longissimus thoracis*; SM: *Semimembranosus*; LL: *L. lumborum*; EC: Electrical Conductivity; JCS: Japanese Color Scale; IMF: Intramuscular fat; NPPC: National Pork Producers Council; Pigments expressed in mg myoglobin/g muscle.

CONCLUSIONS

A first evaluation of the main carcass and meat quality characteristics of the Majorcan Black Pig breed showed that there was a high heterogeneity among the population of the animals studied, as expected due to the lack of selection. The most particular attributes of this breed were high back fat thickness and marbling values (IMF) as well as high pigment content and dark red meat.

Defining the optimal values in the carcass and meat quality variables of this breed, would attain the characteristics of an efficient industrial production system, and achieve a homogeneous and typified final product adapted to consumer demand without losing its traditional feature, with the *sobrassada* as the main product, and the other alternative meat products developed in the future.

So far, carcass and meat quality characteristics of this breed were not seriously considered in the selection of boars and sows. For many years the boars have been selected during popular fairs and events, with the morphological traits as unique criterion. Therefore, a change in the selection program using objective measurements of the carcass and meat quality would be required. This is the first step to reach an economically and environmentally sustainable, as well as efficient production system.

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RIPENING TECHNIQUES AND MICROBIOLOGICAL CHARACTERISTICS OF NEBRODI SALAME

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SUMMARY - With the present work the microbiological and chemical-physical characteristics of salami of the area of "Nebrodi" (Sicily), obtained according to different technical of working/maturation, have been studied. Samples for screening activity collected from several artisanal farms were analysed; these salami were ripened in natural room with different ripening periods. *Salame* (N) produced with meat of "Nebrodi Nero" pork in a local farm and *salame* (I) produced in experimental plant of SSICA (Parma) with meat of "Hybridis" pork were analysed. About sample N high levels of enterobacteria and gram negative bacteria have been found into the mixture. During the different steps, enterobacteria lowered with discontinuity; the development of coagulase positive staphylococci and enterococci has been observed, too. In sample I, the control of hygienic, thermometric and relative humidity conditions allows a continue reduction of pathogenic and spoilage bacteria. The hygienic conditions of working and the techniques of ripening influence the final characteristics of salami.

Key words*:* "Nebrodi" salami, microorganism, technology.

INTRODUCTION

Fermented sausages are the result of biochemical, microbiological, physical and sensorial changes occurring in a meat mixture during ripening under defined conditions of temperature and relative humidity (RH). Traditional fermented food products of meat origin are produced by numerous small-scale and family sized enterprises in Europe.

The preservation of peculiarity of a typical product requires the identification and the quantification of those properties that better describe the characteristics of this product. This could be done by means of microbiological, chemical and sensory characterization. Microbiological and chemical characteristics of typical salami have been extensively studied (Moschetti, *et al.*, 1997, Metaxopoulos *et al*., 1981, Parente, *et al*., 1994, Villani, *et al*. 1994, Diaferia *et al*., 2007, Pirone *et al*., 2007). However, there is a limited number of papers about typical Sicilia salami (Moretti et al., 2004). Salame production in the Nebrodi area (Sicily) is generally carried out according to local tradition. The mixture is executed to "knife tip" using all the cut of pork meat. Salt, entire and milled pepper and natural spices are added to the mixture. Stuffing is carried out in natural pork casing. The ripening is made in natural room, with one first phase of drying that can be executed in air-conditioned rooms. According to the used type of casing and the thermometric and hygrometric conditions, products are obtained with different ripening periods, between 30 to 120 days. With present work, the microbiological characteristics of salame have been studied.

MATERIAL AND METHODS

Product

Samples for screening activity collected from several artisanal farms, *salame* produced with meat of “Nebrodi Nero” pork in a local farm, and *salame* produced in experimental plant of SSICA (Parma) with mixture from meat of “Hybridis” pork were analysed.

Chemical and physicochemical analyses

pH was determined with a CRISON "Micro pH 2001" digital pH meter. Water activity (a_w) was determined using an AQUALAB instrument.

Microbiological analysis

Samples were sliced and slices were then put in a sterile pouch, added with a sterile physiological solution (1:3 ratio) and homogenised with Stomacher for 30 sec.

Micro-organisms and culture media.

Total microbial count: Tryptone soya agar (Oxoid), at 30°C for 72 hours. *Micrococci and* not pathogenic *staphylococci*: Mannitol salt agar (Oxoid), at 37°C for 72 hours. *Staphylococcus* coagulase +: Baird-Parker agar (Oxoid) at 37°C for 48 hours; after isolation, the typical colonies were subjected to coagulase test. *Enterococci*: Kanamycin Aesculin Azide agar Base (Oxoid) at 42°C per 24 hours; the typical colonies were tested with group D antiserum of Streptococcal Grouping Kit (Oxoid). *Enterobacteria and Gram-negatives*: Violet red bile glucose agar (Oxoid), at 37°C for 24 hours for Enterobacteria, + 48 hours at 22°C for total Gram-negatives. *Lactic acid bacteria*: Rogosa agar (Oxoid) at 30°C for 72 hours. *Yeasts and moulds*: Malt extract agar (Oxoid) at 30°C for 72-96 hours.

RESULTS AND DISCUSSION

The activity of screening has regarded 16 samples of *salame* correspondents to 10 different productions. In Table 1 average standard deviation, max and min of *salame* are reported. The level of lactic acid bacteria is always desirable but, at the same time, in a case, the enterobacteria (negative indicators of hygiene) exceeds 10^3 cfu/g. Coagulase positive staphylococci has been found; in one case it is more than 10^4. These is undesirable for the presence of pathogenic bacteria and others studies are necessary regarding the hygiene of the raw materials and correct formulation of production parameters.

Table 1. Samples for screening

(cfu/g)	average	Dev.st	max	min
Total aerobic count	4.0e+08	2.8e+00	1.2e+09	4.8e+07
Gram negative bacteria	1.6e+01	2.5e+01	3.3e+03	<3
Enterobacteria	7.5e+00	1.7e+01	1.5e+03	<3
Micro-staphylococci	3.6e+05	1.4e+01	1.8e+07	2.4e+03
Enterococci	3.5e+03	6.4e+00	9.9e+04	2.1e+02
Stafilococci Coagulase +	7.0e+02	1.8e+01	4.8e+04	<30
Yeast	2.0e+02	3.5e+01	3.0e+04	<30
Moulds	1.5e+01	2.9e+01	2.7e+03	<30
Lactic acid bacteria	3.7e+08	2.5e+00	1.5e+09	7.5e+07

Regarding production artisanal farm of salami made with black pork (Table 2) the mixture shows a good hygienic quality, with low values in enterobacteria, total Gram-negatives bacteria, enterococci and coagulase + staphylococci. Total aerobic count corresponds approximately to concentration in lactic acid bacteria. These grow during the drying so as the not pathogenic micro-staphylococci, but at the same time, also enterococci and coagulase + staphylococci develop. In the next period, until 49 days, growing of enterobacteria and total Gram-negatives bacteria is observed despite the presence of elevated concentrations of lactic acid bacteria. From 50 to 79 days, enterobacteria and total gram negative bacteria are reduced again to low concentrations. Reduction of enterococci and coagulase + staphylococci, instead, has not been found.

Table 2. Black pork *salame*

	Mixture	End-drying	49 days	70 days
Total aerobic count (cfu/g)	3.4E+05	2.4E+08	7.1E+08	8.1E+08
Gram negative bacteria (cfu/g)	3.6E+01	2.1E+01	3.9E+02	<3
Enterobacteria (cfu/g)	3.0E+00	3.0E+00	1.6E+03	<3
Micro-staphylococci (cfu/g)	1.5E+04	1.8E+06	2.1E+06	1.4E+04
Enterococci (cfu/g)	<30	2.4E+04	6.6E+03	4.8E+04
Stafilococci Coagulase + (cfu/g)	<30	9.9E+02	2.7E+04	5.2E+03
Yeast (cfu/g)	1.9E+03	9.9E+02	1.8E+02	5.6E+02
Moulds (cfu/g)	<30	6.0E+01	3.0E+01	4.5E+02
Lactic acid bacteria (cfu/g)	1.3E+05	1.5E+08	6.2E+08	3.9E+08

Regarding the *salame* produced at SSICA, the mixture has a normal microbiological profile with the exception of a concentration of coagulase positive staphylococci (Table 3). At the end of drying a remarkable increase of lactic acid bacteria and not pathogenic micro-staphylococci has been found, according to good ripening; but also a light increase of enterobacteria and gram negative bacteria has been found. These in the successive period decrease, while micro-staphylococci and coagulase positive staphylococci are stable to levels similar to those of end-drying. The product is in good hygienic conditions.

Table 3. Hybrid production at SSICA

	mixture	end drying	end ripening
Total aerobic count (cfu/g)	2.4E+05	8.1E+08	1.2E+09
Gram negative bacteria (cfu/g)	1.3E+03	2.1E+04	1.9E+02
Enterobacteria (cfu/g)	1.2E+03	1.8E+03	<3
Micro-staphylococci (cfu/g)	5.1E+03	1.5E+06	4.8E+05
Enterococci (cfu/g)	<30	<30	1.5E+02
Stafilococci Coagulase + (cfu/g)	6.0E+02	2.4E+02	2.1E+02
Yeast (cfu/g)	1.5E+03	2.6E+03	2.7E+03
Moulds (cfu/g)	<30	<30	<30
Lactic acid bacteria (cfu/g)	6.0E+02	1.4E+08	3.5E+07
pH	5.74	5.52	5.58
aw	0.962	0.949	0.898

CONCLUSIONS

In samples of artisanal production a discreet concentration of enterobacteria and gram negative bacteria has been found in the mixture. The enterobacteria decrease in discontinuous way in the course of the different batch. Presence and following development of pathogenic staphylococci (coagulase positive) and of enterococci has been observed. Lactic acid bacteria and micro-staphylococci grow during all the observed period. In the production carried out at SSICA , the control of the parameters of hygiene, the relative humidity, and of the thermal conditions allow a reduction of the pathogenic and spoilage bacteria.

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MEAT pH INFLUENCE ON TEXTURE OF DRY-CURED HAM WITH REDUCED SALT CONTENT AND SUBMITTED TO DIFFERENT STORAGE TEMPERATURES

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SUMMARY The aim of this study was to determine the effect of raw meat quality on excessive pastiness, adhesiveness and softness of dry-cured ham. Forty-five raw hams were selected according to their pH on the *semimembranosus* (SM) muscle at 24 h post-mortem (Low pH < 5.7; Medium pH = 5.7 ≤ pH ≤ 5.9; High pH > 5.9), covered with dry salt for 10 days (Standard Salting) or 6 days (Reduced Salting) and processed for 12 months in a traditional way increasing the temperatures up to a maximum of 18 ºC ± 2 ºC. At the end of the process they were stored for ten days at 18 ºC or 30 ºC. Thereafter, physicochemical, sensory and instrumental texture analyses were carried out on *biceps femoris* (BF) and SM muscles. Hams with pH_{SM24} < 5.7 are more prone to have texture problems (pastiness, adhesiveness and softness). These problems are greater with Reduced Salting (6 d). Softness in BF muscle was reduced by storing dry-cured hams at 30 ºC for 10 days.

Key Words: Meat quality; NaCl reduction; Storage temperature; Dry-cured ham.

INTRODUCION

Dry-cured ham is a product highly appreciated for its sensory characteristics. Softness and pastiness increase the difficulty in slicing and produce a mouth-coating sensation which could affect consumer acceptability (Arnau, 1991). Softness is associated with high levels of intramuscular and intermuscular fat and low salt to moisture and protein ratios (Parolari et al., 1988), high proteolysis (Parolari et al., 1994; Virgili et al., 1995; García-Rey et al., 2004a), high cathepsin activity (Virgili et al., 1995), high pH (Arnau et al., 1998; Guerrero et al., 1999), low pH (García-Rey et al., 2004b), high water content (Ruiz-Ramírez et al., 2006), seasonality (Virgili & Schivazappa, 2002; García-Rey et al., 2006), maturation conditions (Cilla et al., 2005) and high temperature during the ageing (Arnau et al., 1997). According to Arnau et al. (1997) a high temperature, during the last month of ageing, decreases hardness and increases pastiness of the *biceps femoris* (BF) muscle in dry-cured hams aged for six months. However, Morales et al. (2007a) showed that samples of BF dry-cured muscles stored at 30 ºC during the last month of ageing were harder than those stored at 5 ºC, despite showing a higher proteolysis index. In this sense, Morales et al. (2007b) reported that the thermal treatment at 30 °C for 168 h on sections of dry-cured hams decreased the softness, adhesiveness and pastiness in BF muscle significantly, without increasing the hardness in *semimembranosus* (SM) muscle and without affecting the moisture, a_w and proteolysis index. However, no study of the effect of the temperature during the last days of ageing in dry-cured hams has been carried out.

The aim of this study was to determine the effect of raw meat quality on excessive pastiness, adhesiveness and softness of dry-cured ham.

MATERIAL AND METHODS

Ham selection and dry-curing process

Forty-five raw hams ranging from 11 kg to 13 kg were selected in a commercial slaughterhouse according to the pH measured on the SM muscle at 24 h post-mortem (pH_{SM24}): 16 hams with pH_{SM24} < 5.7, 14 hams with pH_{SM24} between 5.7 and 5.9, and 15 hams with pH_{SM24} >5.9. The pH was measured with a pH penetration electrode (Crison 52-32) on a portable pH-meter (Crison pH 25, Crison Instruments, S.A, Alella, Spain).

All the hams were rubbed at 36 hours post-mortem with 0.5 g KNO_3, 0.3 g $NaNO_2$, 0.5 sodium ascorbate and 10 g of NaCl per kg of raw ham. Subsequently, hams were covered with dry salt and piled up at 3 ºC ± 2 ºC for 6 d (Reduced Salting) or 10 d (Standard Salting) depending on the salting treatment.

After salting, the hams were washed with cold water and hung in a drying room at 2 ºC ± 2 ºC and 78 % ± 2 % RH for 62 days. Afterwards, the hams were dried for 34 d at 11 ºC ± 2 ºC and 70 % – 80 % RH; 10 d at 12 ºC ± 2 ºC and 60 % – 75 % RH; 106 d at 14 ºC ± 2 ºC and 50 % – 65 % RH; and 126 d at 18 ºC ± 2 ºC and 40 % – 55 % RH. The lean surface of the hams that reached a weight loss of 28.5 % was covered with a layer of melted fat to slow down the drying rate. Ham weight losses were monitored until achieving a final weight loss of 33 %. During the last 10 d of the process 22 hams were stored at 18 ºC ± 2 ºC and 40 % – 55 % RH and the rest were stored at 30 ºC ± 2 ºC and 40 % – 55 % RH.

Sample preparation

Hams were cut at the head of the femur level, and two sections of 20 mm perpendicular to the femur axis were obtained from the distal part. The first section was used for instrumental texture and physiochemical analyses. The adjacent section was used for sensory analysis.
Five specimens from SM and BF muscles of the first section were accurately carved with a scalpel into parallelepipeds of 20 mm × 20 mm × 15 mm (length × width × height). The specimens were wrapped in film to avoid drying, packed in PA/PE bags with a water permeability of 2.6 $g/m^2/d$ at 23 ºC and 85 % RH (SACOLIVA®, S.L., Castellar del Vallès, Spain) and stored for 24 h at 4 ºC ± 2 ºC before the instrumental texture analysis. The trimmings from each muscle were ground, vacuum-packed and stored at -20 ºC ± 2 ºC for further physicochemical analyses: total nitrogen, non-protein nitrogen content, NaCl and intramuscular fat contents.

Texture analysis

A Stress Relaxation (SR) test was performed on all the samples using a Universal Texture Analyser TA.TX2 (Stable Micro Systems Ltd., Surrey, England) with a 25 kg load cell and a 50 mm diameter compression plate. The specimens were compressed 25 % of their original height, perpendicular to the fibre bundle direction and at a crosshead speed of 1 mm/s. The force versus time after the compression was recorded at a speed of 50 points per second for 90 s (relaxation time). The relaxation curves obtained for each specimen were normalized, i.e., the force decay Y(t) was calculated as follows:

$$Y(\mathrm{t}) = \frac{F_0 - F(\mathrm{t})}{F_0} \qquad (1);$$

where F_0 (kg) is the initial force and F(t) is the force recorded after t seconds of relaxation. The force decay at 2 s (Y_2) and 90 s (Y_{90}) were calculated (Morales et al., 2007c). The average of the five specimens per sample was used for statistical analysis. After the SR tests the specimens used were minced, vacuum-packed and kept at 2 ºC ± 2 ºC for a_w and moisture analysis.

Sensory texture analysis

The sensory analysis was performed on 1-mm-thick slices by a six-member expert panel in 16 sessions and separately for SM and BF muscles. The assessors were selected and generically trained following ISO standards. In each session the order of presentation and the first-order carry-over effect were blocked (Macfie et al., 1989). The average scores of the panel for each sample were used for statistical analysis. Texture (hardness, pastiness, crumbliness, adhesiveness and fibrousness) descriptors were quantified using a non-structured scale ranging from 0 (absence) to 10 (maximum intensity).

Physicochemical analysis

Water activity (a_w) was measured at 25 ºC ± 0.3 ºC with a Novasina AW-SPRINT-TH 500 instrument (Axair Ltd., Pfäffikon, Switzerland). After measuring a_w, the water content of the samples was immediately determined by drying at 103 ºC ± 2 ºC until reaching constant weight (AOAC, 1990). The NaCl content was measured using a Technicon™ AutoAnalyzer™ II (Bran+Luebbe GmbH, Norderstedt, Germany) based on the photometric method described by Zall et al. (1956). The total IMF was measured by Soxhlet extraction (ISO 1443:1973). The total nitrogen (TN) was measured by the Kjeldahl method (ISO 937:1978) and the non-protein nitrogen content (NPN) by precipitation of proteins with trichloroacetic acid (Gáspár, 1984) followed by determination of the nitrogen in the extract by the Kjeldahl method. A proteolysis index (PI %) was calculated as the ratio 100 × (NPN/TN) (Careri et al., 1993).

Statistical analysis

The analyses of variance were performed with the General Linear Model (GLM) procedure of the SAS statistical package (SAS Institute, 2001). The model included pH group, salting time, storage temperature and their interactions as fixed effects. The analyses were carried out by muscle (SM and BF). The interactions not statistically significant ($P > 0.05$) were dropped from the models. Differences among means within each effect were tested by the Tukey test.

RESULTS AND DISCUSION

Physicochemical analysis

No significant interactions were observed and they were dropped from the model. No significant effects of pH_{SM24} on physicochemical parameters were found. Those physicochemical parameters of SM and BF muscles significantly affected by salting time and storage temperature are shown in Table 1. As was expected, the NaCl content decreased and the a_w and the PI increased significantly ($P < 0.05$) in both BF and SM muscles when the salting time was reduced from 10 d to 6 d. Similar results on PI were found in previous studies (Arnau et al., 1998; Ruiz-Ramírez et al., 2005; Morales., 2007a). These results could be explained by the inhibitory effect of NaCl on protease activities (Sárraga et al, 1989; Rico et al., 1990). Intramuscular fat and protein contents were not statistically different.

Table 1. Physicochemical parameters (least square means) significantly affected by salting time and/or storage temperature

	Salting time		Storage temperature		RMSE
	6 d	10 d	18 ºC	30 ºC	
Semimembranosus					
a_w	0.909[a]	0.901[b]	0.906	0.904	0.0116
Proteolysis index (%)	19.5[a]	17.5[b]	17.6[b]	19.4[a]	3.21
NaCl (g/100g DM)	6.88[b]	9.63[a]	8.52[a]	7.99[b]	2.071
Biceps femoris					
a_w	0.933[a]	0.914[b]	0.921	0.926	0.0096
Proteolysis index (%)	28.3[a]	24.6[b]	24.6[b]	28.3[a]	3.86
NaCl (g/100g DM)	11.38[b]	14.10[a]	8.52	7.99	2.335

[ab] Within factor and row, means with different letters are significantly different ($P < 0.05$)
RMSE: root mean square error

The PI was higher in samples stored at 30ºC for 10 days than in those stored at 18 ºC. Similarly, Morales et al. (2007a) found that the PI was higher in BF muscles aged for 30 days at 30 ºC than in those aged at 5 ºC. This temperature effect on PI has also been widely described in previous studies (Arnau, 1991; Arnau et al., 1997; Parolari et al., 1994; Virgili et al., 1995). The temperature promotes the activities of cathepsin B and L (Rico et al., 1990; Zhao et al., 2005), thereby increasing the PI. In contrast, Morales et al. (2007b) did not find any significant differences of PI in dry-cured ham dices and dry-cured ham pieces (4 cm-thick) treated at 30 ºC for only 7 days.

Instrumental and sensory texture

Table 2 shows the LSMeans of Stress Relaxation (SR) test parameters and sensory descriptors of BF muscle significantly affected by pH_{SM24} group, salting time or storage temperature. The SR test parameters in SM muscle were not affected significantly by the different fixed effects. In order to understand the SR test parameters better it can be assumed that low F_0 and high force decay at 2 s (Y_2) and at 90 s (Y_{90}) indicate a high softness (Morales et al. 2007c).

Table 2. Stress Relaxation test parameters and sensory texture descriptors (least square means) of *biceps femoris* muscle significantly affected by pH_{SM24} (LpH: $pH_{SM24} < 5.7$; MpH: $5.7 < pH_{SM24} \leq 5.9$; HpH: $pH_{SM24} > 5.9$), salting time or storage temperature

	pH_{SM24} group			Salting time		Storage temperature		RMSE
	LpH	MpH	HpH	6 d	10 d	18 ºC	30 ºC	
F_0	0.910^b	1.024^{ab}	1.170^a	1.030	1.040	0.899^b	1.170^a	0.3428
Y_2	0.350^a	0.336^{ab}	0.318^b	0.337	0.333	0.350^a	0.320^b	0.0273
Y_{90}	0.710^a	0.705^{ab}	0.688^b	0.706	0.696	0.729^a	0.673^b	0.0241
Pastiness	2.2	2.1	1.2	2.6^a	1.0^b	2.0	1.6	1.08
Adhesiveness	3.3	3.5	2.4	3.9^a	2.3^b	3.5^a	2.7^b	1.10
Hardness	3.0	3.0	3.2	2.8^b	3.3^a	3.1	3.0	0.29

[ab] Within factor and row, means with different letters are significantly different ($P < 0.05$)
RMSE: root mean square error

The LpH group had lower F_0 and higher Y_2 and Y_{90} than HpH group (Table 2). This result could probably be explained by a higher PI, although differences were not significant. Whereas, high pH values have also been related with softness by Morales et al. (2007a) in dry-cured BF muscles aged for one month (pH_{SM24} values from 6.1 to 7.0), and by Arnau et al. (1998) and Guerrero et al. (1999) in dry-cured hams ($pH_{SM24} \geq 6.2$). The pH_{SM24} of the hams from our HpH group ranged from 5.9 to 6.2 and it seems that this was not high enough to increase the softness in the final product. In addition, Ruiz-Ramírez et al. (2006) found that the hardness (Texture Profile Analysis) was lower in samples with low pH ($pH_{SM24} < 5.7$) than those with $pH_{SM24} > 6.2$ and this difference was related with higher proteolytic activity in the low pH samples. García-Rey et al. (2004b) related low pH with softness in dry-cured ham as a result of higher proteolysis activity. High proteolytic activity has been suggested as the main cause of soft and pasty texture in dry-cured ham by several authors (Arnau, 1991; Arnau et al., 1997; Careri et al., 1993; Virgili et al., 1995).

F_0 values were higher and Y_2, Y_{90} and adhesiveness lower in BF muscles stored at 30 ºC ($P <$ 0.05) than in those stored at 18 ºC (Table 2). In this context, Martínez et al. (2007) found that dry-cured hams aged at 32 ºC – 34 ºC for 30 days at the end of the process showed greater hardness (Texture Profile Analysis) and lower moisture content than those aged at 23 ºC – 24 ºC for 60 days. However this study could not discriminate between temperature and moisture content effects. Morales et al. (2007a) found that BF dry-cured muscles stored at 5 ºC had lower F_0 and higher Y_2 than those stored at 30 ºC. Similarly, Morales et al. (2007b) reported that dry-cured ham pieces stored at 30 ºC for 168 h were harder than those stored at 4 ºC.

Adhesiveness and pastiness (BF muscle) were lower with standard salting than with reduced salting whereas hardness was higher with standard salting. Similarly, Andrés et al., (2004) found that salt level affected the hardness and the fibrousness in Iberian dry-cured ham positively. As mentioned previously, NaCl content has an inhibitory effect on the proteolytic activity and could also cause a compression of myofibril proteins, which would reduce the softness (Shomer et al., 1987). This could explain the greater hardness and lower pastiness that the hams at standard salting showed.

The interaction pH_{SM24} × salting time was significant (P < 0.05) for pastiness and hardness in SM muscle (Table 3). Within the LpH group, the SM muscles salted for 6 d showed lower hardness and higher pastiness than those salted for 10 d (P < 0.05). Within hams salted for 6 days, the pastiness was lower in HpH group than in LpH group. These results indicate that the effect of decreasing salting time on pastiness was higher when the pH was < 5.7.

Table 3. Hardness and pastiness of *semimembranosus* muscle according to the interaction pH_{SM24} (LpH: pH_{SM24} < 5.7; MpH: 5.7 < pH_{SM24} ≤ 5.9; HpH: pH_{SM24} > 5.9) × salting time (least square means)

		pH_{SM24} group		
	Salting time	LpH	MpH	HpH
Semimembranosus				
Pastiness	6 d	2.9^{ax}	1.7^{ab}	0.4^{b}
(RMSE=1.02)	10 d	0.4^{y}	0.5	0.5
Hardness	6 d	4.0^{y}	4.5	4.7
(RMSE=0.29)	10 d	5.7^{ax}	4.3^{b}	5.1^{ab}

^{abc} Within row, means with different letters are significantly different (P < 0.05)
^{xy} Within column, means with different letters are significantly different (P < 0.05)
RMSE: root mean square error

CONCLUSIONS

Hams with pH_{SM24} < 5.7 are more prone to have texture problems (pastiness, adhesiveness and softness), especially at short salting times. Softness in BF muscle was reduced by storing the dry-cured hams at 30 ºC for 10 days.

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EVOLUTION OF QUALITY OF IBERIAN BACON PACKAGED UNDER MODIFIED ATMOSPHERE

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SUMMARY-. The objective of this research was to study the effects of different concentrations of carbon dioxide and the presence of low levels of carbon monoxide throughout storage of Iberian bacon. Iberian bacon slices were packaged under modified atmospheres of 20%CO_2/80%O_2 and 30%CO_2/69.9%Ar/0.1%CO. The packs were stored at 4ºC for up to 14 days. Colour, odour and acceptance were assessed by a trained sensory panel. Colorimetric parameters, pH, instrumental texture and microbial quality were also analysed. No statistically differences ($p>0.05$) among atmospheres types were found in the colorimetric parameters, instrumental texture and microbial counts. Regarding pH, the values were lower at the end of the storage time in samples packed under 30%CO_2/69.9%Ar/0.1%CO. Results obtained from sensory analysis showed that the both atmospheres maintained the colour for 14 days, but samples packed under 30%CO_2/69,9%Ar/0,1%CO_2 presented off-odours for up to 7 days. This fact involved that the judges failed these samples.

Key words: bacon, modified atmospheres, monoxide carbon, storage quality.

INTRODUCTION

The main reasons for modified atmosphere packaging (MAP) of meats for retail sale are to prolong the microbiological shelf life and to maintain an attractive red colour of product. MAP usually includes CO_2 and O_2. CO_2 inhibits the growth of many microorganisms but high concentrations (over 40%) may result in brown discoloration on meat surfaces (Silliker *et al.*, 1977). Inclusion of O_2 concentration (70-80%) allow meat to bloom to the bright red (Wicklund *et al.*, 2006), however O_2 support the growth of spoilage bacteria (Sorheim *et al.*, 1999) and favours oxidative reactions. Oxidation of myoglobin to metmyoglobin results in a brown, unacceptable discolouration of the meat. In addition, O_2 promotes lipid oxidation and the development of off-odours and flavours (Jayasingh *et al.*, 2002). In order to reduce these disfavourable effects, the O_2 may be removed by vacuum and/or displaced by an inert gas. Argon has been used in MAP in order to displace and exclude O_2 more efficiently than N_2 and provide better control against oxidation of flavour and colour components (Spencer, 2005). In other hand, because metmyoglobin forms rapidly at the surfaces of meats exposed to low concentrations of O_2, addition of CO to the MAP gas mixture had been proposed to preserve fresh meat colour (Sørheim *et al.*, 1999; Luño *et al.*, 2000, Martínez *et al.*, 2005; Wicklund *et al.*, 2006, Wilkinson *et al.*, 2006).

The aim of this work was to study the effects of a commercial CO_2/O_2 gas mixture and an alternative MAP (with argon and CO) on the sensory characteristics and microflora of bacon sliced, packed and stored at 5ºC for up to 14 days.

MATERIAL AND METHODS

In this study, bacon fresh slices from Iberian pig were packaged in two modified atmospheres: 20%CO_2/80%O_2 and 30%CO_2/69.9%Ar/0.1%CO. The slices were packaged in polystyrene rigid trays which, after flushing with the selected gas mixture, were closed by heat-sealing with a packer (Linvac 400 TECNOVAC, Barcelona, Spain) in a high barrier film (with an oxygen transmission rate of 1.8 $cm^3/m^2/24h/bar$ at 20ºC and 65% RH, supplied by Fibosa Packaging S.L., Tordera, Spain). The trays were stored in darkness at 5ºC and the packs were opened for subsequent analysis after 0, 3, 7, 10 and 14 days.

Sensory evaluation

Sensory evaluation was carried out on bacon slices after each storage time by an experienced 8-member sensory panel. The attributes were divided into visual aspects (fat and lean colour and brightness), off-odour (which was evaluated between 0.5-1 min after opening of the packs) and acceptance. They were scored on a 5-point scale: 1= minimum intensity, 5= maximum intensity. The colour was assessed on a scale where 1= red (lean)/ white (fat) and 5= brown (lean)/ yellow (fat). Scores below 3 were considered no acceptable, except for colour and off-odour that were considered no acceptable when the score was higher than 3.

Microbiological analysis

The samples were analysed for psychrotrophic bacteria (Plate Count Agar (Scharlau, Spain) at 7ºC for 10 days), Enterobacteriaceae (3M Petrifilm Enterobacteriaceae Count Plate (Bioser, Barcelona, Spain) at 37ºC for 24h), lactic acid bacteria (LAB) (MRS Agar (Scharlau, Spain) at 30ºC 72 h) and Pseudomonads (Pseudomonads Agar (Oxoid, Spain) supplemented with Cetrimide, Fucidine and Cephaloridine (CFC) (Oxoid, Spain), at 30ºC for 48 h).

Physic-chemical analysis

The pH values were determined by blending 10 g of product with 10 ml of distilled water for 2 min, with a pH meter model 507 (Crison Instruments, Barcelona, Spain) equipped with a glass electrode and setting of temperature. Surface colour of bacon slices was measured using a reflectance spectrophotometer (Minolta CM-2002; Osaka, Japan). The illuminant used was D65 (colour temperature of 6504 K) and the standard observer position was 10º. Colour results were determined in the CIE-LAB system and the lightness (L*), redness (a*, red↔green) and yellowness (b*, yellow↔blue) were calculated. Finally, samples of cooked bacon (1x1x3.5cm) were tested by the Warner-Bratzler test using a texture analyzer TA-XT2 (Stable Micro Systems, Haslemere, UK). The crosshead speed was set to 50 mm/min and the shear force was measured and expressed in g. The Stable Micro Systems Texture Expert, version 1.20 (Spanish), computer program was used for data collection and calculations.

Statistical analysis

The factors analysed were type of packaging with two levels and days of storage with five levels. The significance of days of storage and packaging method was determined using one-way analysis of variance (ANOVA). The means were separated by Tukey-honest significant difference test at 5% level. Data analyses were conducted using the statistical package STATISTICA 6.0.

RESULTS AND DISCUSSION

Table 1 show the results obtained on the sensory evaluation. Colour fat scores indicated no differences between the two atmospheres evaluated until the end of storage. At the 14th day, a slight yellow colour was observed in the fat of the 20%/80% CO_2/N_2 packaged samples. Regarding lean colour, at 0 day bacon packed with 30%CO_2/69.9%Ar/0.1%CO was considered unacceptable due to the slightly brown lean colour. This result was due to that the visual colour evaluation was carried out immediately after packaging, before CO is going to bind with myoglobin to form the carboxymyoglobin. So, in the others sampling times the packs maintained the bright red colour characteristic of carboxymyoglobin. On the contrary, bacon slices packed in 20%CO_2/80%O_2 discoloured during storage and had scored of 3 (limit of acceptable colour) at the 10th day of storage. Additionally, visual assessment indicated that the bacon slices in CO MAP were visually more attractive than those packaged in high O_2 MAP.

Table 1. Effect of packaging conditions (20%CO_2/80%O_2 and 30%CO_2/69.9%Ar/0.1%CO) on sensorial parameters on bacon slices stored at 5ºC during 14 days.

Parameters	Packaging	0 days	3 days	7 days	10 days	14 days
Fat colour *	20% CO_2 80% O_2	1	1	1	1	3
	30% CO_2 69.9% Ar 0.1% CO	1	1	1	1	1
Lean colour *	20% CO_2 80% O_2	1	1	2	3	4
	30% CO_2 69.9% Ar 0.1% CO	4	1	1	2	2
Brigthness	20% CO_2 80% O_2	5	5	4	3	1
	30% CO_2 69.9% Ar 0.1% CO	5	4	4	3	3
Off-odour	20% CO_2 80% O_2	1	2	2	3	4
	30% CO_2 69.9% Ar 0.1% CO	1	3	4	5	5
Acceptance	20% CO_2 80% O_2	5	4	3	3	2
	30% CO_2 69.9% Ar 0.1% CO	2	4	2	2	1

1= minimum intensity, 5= maximum intensity.
*Colour scale: 1= red (lean)/ white (fat) and 5= brown (lean)/ yellow (fat).

Off-odour scores showed that it increased throughout storage in all samples. The shelf-life of the bacon, as determined by the time to the develop off-oddours, was influenced by the packaging method. Off-oddours were not acceptable 7 days earlier for bacon slices stored in CO than for bacon slices stored in 20%CO_2/80%O_2. However, no effect of MAP treatment was observed for any of the microorganisms studied. These results don´t agree with those of Sorheim *et al.* (1999) who found that off-oddours developed more slowly in meat packed with CO than in meat packed in high O_2 MAP. Besides, these authors pointed out that the growth of pseudomonads is retarded under anaerobic conditions and that the high CO_2 concentrations and absence of O_2 could favour the growth of LAB.

Results of the pH, colour and texture instrumental measurements are shown in Table 2. Values for pH ranged from 6.1 to 6.5 and were lower at the end of the storage time in samples packed under 30%CO_2 /69.9%Ar/0.1%CO. This effect could be due to the higher CO_2 concentration in this mixture Other authors (Martínez *et al*., 2005) reported that increasing CO_2 concentration gave rise to a lowering of pH due to the dissolution of CO_2 into the product which resulted in the production of carbonic acid.

With regard to the colour parameters, the packaging system had little influence on L* and no differences were found between bacon packed with 30%CO_2/69.9%Ar/0.1%CO and 20%/80% CO_2, except at day 0 of storage. Besides, significant differences ($p<0.05$) were found between 0 and 3 days in samples packed with 30%CO_2/69.9%Ar/0.1%CO.

Table 2. Effect of packaging conditions (20%CO_2/80%O_2 and 30%CO_2/69.9%Ar/0.1%CO) on pH, instrumental colour and texture parameter (mean±S.D.) on bacon slices stored at 5ºC during 14 days.

Parameters	Packaging	0 days	3 days	7 days	10 days	14 days
pH	20% CO_2 80% O_2	^{a}6.5±0.1^{A}	^{a}6.5±0.1^{B}	^{a}6.4±0.1^{A}	^{a}6.5±0.1^{B}	^{a}6.5±0.0^{B}
	30% CO_2 69.9% Ar 0.1% CO	^{b}6.5±0.1^{A}	^{ab}6.3±0.1^{A}	^{a}6.2±0.0^{A}	^{ab}6.3±0.0^{A}	^{a}6.1±0.1^{A}
L*	20% CO_2 80% O_2	^{ab}73.4±0.4^{B}	^{ab}70.8±0.4^{A}	^{b}73.3±0.5^{A}	^{b}78.0±0.3^{A}	^{a}65.7±3.2^{A}
	30% CO_2 69.9% Ar 0.1% CO	^{ab}60.7±5.4^{A}	^{c}77.7±0.9^{A}	^{bc}74.7±1.5^{A}	^{bc}72.1±0.7^{A}	^{c}67.4±5.2^{A}
a*	20% CO_2 80% O_2	^{a}2.0±0.1^{A}	^{b}5.1±0.2^{B}	^{ab}3.4±0.6^{A}	^{a}1.6±0.1^{A}	^{b}6.4±1.7^{A}
	30% CO_2 69.9% Ar 0.1% CO	^{ab}2.7±1.1^{A}	^{a}1.8±0.3^{A}	^{ab}2.9±1.0^{A}	^{bc}4.6±0.2^{B}	^{c}5.4+1.2^{A}
b*	20% CO_2 80% O_2	^{ab}10.3±0.7^{A}	^{c}13.0±0.1^{B}	^{ab}10.5±1.3^{A}	^{a}8.8±0.3^{A}	^{bc}11.8±1.0^{A}
	30% CO_2 69.9% Ar 0.1% CO	^{ab}9.5±1.0^{A}	^{a}9.0±0.9^{A}	^{ac}10.8±1.2^{A}	^{c}12.4±0.4^{B}	^{bc}11.7±0.6^{A}
Shear force (g)	20% CO_2 80% O_2	^{a}2.2±0.1^{A}	^{a}2.3±0.5^{A}	^{a}2.3±0.1^{A}	^{a}2.5±0.3^{A}	^{a}2.5±0.4^{A}
	30% CO_2 69.9% Ar 0.1% CO	^{a}2.4±0.2^{A}	^{a}2.3±0.3^{A}	^{a}2.2±0.4^{A}	^{a}2.0±0.2^{A}	^{a}2.3±0.2^{A}

a-c: Averages with different letter in the same row are different ($p<0.05$).
A-B: Averages of each parameter with different letter in the same column are different ($p<0.05$).

Redness (a*) values, which is used as an indicator of colour stability in meat, showed an increase at the end of the storage time (Table 2) in the two MAPS evaluated. Although the lean in the bacon slices packed under 30%CO_2 /69.9%Ar/0.1%CO were redder than those under 20%CO_2/80%O_2 mixture (Table 1), no clear differences were observed in a* values (Table 2). Several authors (Sørheim *et al.* 1999, Luño *et al.* 2000, Jayasingh *et al.* 2001, Krause *et al.* 2003, John *et al.* 2005, Martínez *et al*. 2005) found that a* values were significantly higher in MAP containing CO than in traditional aerobic packages on fresh meat and different meat products. Respect to the yellowness (b*), statistical differences ($p<0.05$) were found at some specific sampling times and between packaging methods at 3 and 10 days. However, these differences did not allow us to establish a clear difference between packaging systems. Finally, no statistical differences were found in texture evaluation (Table 2) during storage and between MAPS.

CONCLUSIONS

On the basis of the results obtained in this work, it can be concluded that the use of carbon monoxide into the packs of bacon slices increased the amount of time during which the product was considered visually acceptable, however the shelf-life was not increased in relation to the habitual MAP.

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EFFECTS OF HIGH PRESSURE TREATMENT ON THE QUALITY OF VACUUM-PACKED "SALCHICHÓN" ENRICHED IN MONOUNSATURATED FATTY ACIDS

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SUMMARY - In this study, two different types of dry fermented *salchichón* were manufactured with meat pork and backfat pork from pigs fed with different diets (control diet and high oleic diet). They were sliced, vacuum packed and treated with high hydrostatic pressure (500 MPa, 5 min) and further stored at 6ºC for up to 210 days. The objective was to compare the microbiological and sensory evolution of the high pressure processed samples and the untreated samples of both sausages (control *salchichón* and high oleic *salchichón*). Taking into account the results obtained, high pressure treatment had an inhibitory effect on some groups of microorganisms, especially on psychrotrophic, lactic acid bacteria and yeast and moulds. Consequentially, microbial counts fell, although injured microorganisms recovered during storage except in the case of the yeast and moulds counts. Besides, high-pressure treatment had no noticeable effect on sensory properties of the samples. For that, these results suggest that HPP is a suitable preservation method with no detrimental effects on organoleptic properties of salchichón.

Key words: vacuum packaging, high pressure processing, fermented sausage, salchichón, meat product.

INTRODUCTION

Salchichón is one of the most important dry-cured sausages produced in Spain. The basic ingredients of *salchichón* are lean pork, pork backfat, salt and spices. Pork meat has often been blamed for being too high in fat, especially in saturated fat. Consumption of these meat products marketed after slicing has increased over recent years, despite concerns over their high content of animal fats. For that, the meat industry is trying to address this problem through modification of the lipid fraction, by increasing the percentages of monounsaturated fatty acids (MUFA). Besides, packaged products require longer shelf lives, and should be resistant to microbial spoilage, which has led to ongoing research into new technologies to preserve the products high nutritional and sensory qualities and their comparability with similar untreated products, whilst assuring microbiological safety over a long shelf life. High-Pressure Processing (HPP) is one of the most promising technologies in the treatment of sliced cured meat products. The aim of this work was to study the microbiological and sensory evolution of two types of sausages differing in fat composition (control *salchichón* and high oleic *salchichón*) treated with high pressure during long chilled storage time (210 days) and to determine if HPP is a valid preservation method to increase the *salchichón* safety without noticeable changes on its quality properties during wide chilled storage.

MATERIAL AND METHODS

The sausages were manufactured according to a traditional formulation with lean and backfat obtained of pigs fed with different diets: 1) control (CO) and 2) enriched in monounsaturated fatty acids (HO). Two sausages, randomly selected for each batch, were sliced at 1 mm thickness and 100 g of slices were placed in polystyrene trays. Then, the trays were introduced in plastic bags (polyamide/polyethylene), which were subjected to vacuum and sealed. Following vacuum packing, the samples were treated under high pressure, except for one group from each batch (CO, HO) that remained untreated. HPP was performed at an industrial hydrostatic pressure unit equipped with a 135 l volume high-pressure vessel using additive-free water as the pressure transmitting fluid. The pressure level was set at 500 MPa, treatment time at 5 min and the initial water temperature at 18 °C. The samples treated with high pressure (HP) and the untreated samples (UT) were stored at 6 °C for up to 210 days. Microbiological and sensory analyses were performed on two packs taken from each batch (CO, HO) at selected times: before high pressure processing (0 day), after high pressure processing (1 day) and during storage (15, 30, 60, 90, 150 and 210 days).

Microbiological analysis

The samples were analysed for psychrotrophic bacteria (Plate Count Agar (Scharlau, Spain) at 7ºC for 10 days), *Enterobacteriaceae* (3M Petrifilm Enterobacteriaceae Count Plate (Bioser, Barcelona, Spain) at 37ºC for 24h), lactic acid bacteria (LAB) (MRS Agar (Scharlau, Spain) at 30ºC for 72 h), *Micrococcaceae* (MSA (Scharlau, Spain) at 37ºC for 48 h), yeasts and moulds (3M Petrifilm Yeast and Mold Count Plate (Bioser, Barcelona, Spain) at 25ºC for 5 days).

Sensory evaluation

Sensory evaluation was carried out on *salchichón* slices after each storage time by an experienced 8-member sensory panel. The sensory attributes (colour, odour, taste, hardness, juiciness and overall acceptability) were scored using 5-point scales (5 = excellent, 4 = good, 3 = acceptable, 2 = fair and 1 = unacceptable).

Statistical analysis

Data collected for microbiology and sensory attributes were statistically analysed by a three-factor factorial arrangement. The factors were the two *salchichón* types (control, and high oleic), the two treatments (UT and HP samples) and the storage time (0, 1, 15, 30, 60, 90, 150 and 210 days). The data were analyzed by analysis of variance (ANOVA). When main effects were significant, the means were separated by Fisher's least significant difference test at 5% level ($LSD_{0.05}$). The level of significance $p<0.05$ was used for all comparisons and will be used for the remainder of this discussion. Data analyses were conducted using the statistical package Statgraphics Plus 5.0.

RESULTS AND DISCUSSION

Table 1 shows the microbiological results obtained. *Salchichón* type significantly ($p<0.05$) affected the microbial numbers except for *Micrococcaceae*. The lowest counts were found on CO *salchichón*. The differences observed between *salchichón* groups could be due probably to the antibacterial activity of fatty acids. Authors as Branen *et al.* (1980) and Rubio *et al.* (2007) found that saturated fatty acids had the greatest influence on antibacterial activity.

High-pressure treatment had significant effect ($p<0.05$) on counts of different microorganism groups and the HP samples presented counts below those obtained in the UT samples. *Micrococcaceae* numbers were hardly affected. That agrees with some authors who state that microorganism sensitivity to HPP is related to cell morphology; cocci being the most pressure resistant (Mor-Mur and Yuste, 2005). Throughout the storage period, all microbial numbers decreased slowly and this pattern was similar to the evolution reported by Rubio *et al.* (2007) in vacuum-packed *salchichón*.

The sensorial characteristics studied on the two groups of sausages, are reported in Table 2. Differences ($p<0.05$) were found between sausage groups except for odour. However, no differences ($p>0.05$) were found between treatments except for acceptability and the analytical results showed that sensory attributes slightly varied after HPP treatment (1 day). Storage time had significant effects ($p<0.05$) on sensory parameters considered and the sensory parameters values decreased linearly along time.

Table 1. Effect of *salchichón* type, treatment and storage time on microbiological parameters of *salchichón* stored at 6ºC.

	Psychrotrophs	Lactic acid bacteria	*Micrococcaceae*	Yeasts and moulds
Salchichón type[A]				
CO	7.31[a]	7.81[a]	3.48	2.22[a]
HO	7.65[b]	8.04[b]	3.57	3.33[b]
$LSD_{0.05}$	0.17	0.16	0.09	0.40
Treatment				
UT	7.82[b]	8.22[b]	3.61[b]	3.55[b]
HP	7.15[a]	7.63[a]	3.45[a]	2.00[a]
$LSD_{0.05}$	0.17	0.16	0.09	0.40
Storage Time				
0	7.57[b]	8.64[c]	3.72[d]	5.02[d]
1	6.91[a]	8.13[b]	3.70[cd]	3.41[c]
15	7.73[bc]	8.11[b]	3.53[bc]	3.56[c]
30	7.98[c]	8.00[b]	3.56[bcd]	2.57[b]
60	7.83[bc]	7.89[b]	3.38[b]	1.88[ab]
90	7.97[c]	7.87[b]	3.62[cd]	2.01[ab]
150	6.98[a]	7.33[a]	3.54[bcd]	1.67[a]
210	6.88[a]	7.43[a]	3.17[a]	2.08[ab]
$LSD_{0.05}$	0.35	0.31	0.19	0.79

[a,b,c,d] Means within the same column and the same main effect with different superscript letters are different ($p<0.05$).

Table 2. Effect of *salchichón* type, treatment and storage time on sensory parameters of *salchichón* stored at 6ºC. Values rated on a 5 point scale (5 = excellent, 4 = good, 3 = acceptable, 2 = fair and 1 = unacceptable).

	Colour	Odour	Taste	Hardness	Juiciness	Acceptability
Salchichón type						
CO	4.16[a]	3.93	3.70[a]	3.66[a]	3.73[a]	3.70[a]
HO	4.30[b]	3.96	3.85[b]	3.93[b]	4.05[b]	3.90[b]
$LSD_{0.05}$	0.12	0.11	0.12	0.14	0.13	0.10
Treatment						
UT	4.21	3.90	3.74	3.76	3.85	3.75[a]
HP	4.24	3.99	3.80	3.83	3.92	3.85[b]
$LSD_{0.05}$	0.12	0.11	0.12	0.14	0.13	0.10
Storage Time						
0	4.88[f]	4.63[e]	4.75[f]	4.88[e]	4.97[f]	4.81[f]
1	4.70[ef]	4.63[e]	4.48[e]	4.18[d]	4.28[e]	4.25[e]
15	4.48[de]	4.14[d]	4.02[d]	3.85[c]	4.06[de]	3.88[d]
30	4.51[e]	4.09[d]	3.93[d]	3.67[bc]	3.84[cd]	3.85[d]
60	4.26[d]	3.75[c]	3.63[c]	3.71[bc]	3.87[cd]	3.73[cd]
90	3.68[b]	3.70[bc]	3.50[bc]	3.60[bc]	3.57[b]	3.59[c]
150	3.98[c]	3.51[b]	3.27[b]	3.48[b]	3.71[bc]	3.37[b]
210	3.31[a]	3.10[a]	2.60[a]	2.99[a]	2.80[a]	2.91[a]
$LSD_{0.05}$	0.24	0.23	0.23	0.27	0.25	0.19

[a,b,c,d,e,f] Means within the same column and the same main effect with different superscript letters are different ($p<0.05$).

CONCLUSIONS

Taken together these results indicate that high pressure treatment had a slight inhibitory effect on some groups of microorganisms and HPP did not determine clear differences in the sensory properties of the *salchichón*, even though this product is rich in monounsaturated fatty acids. These results suggest that HPP is a suitable preservation method with no detrimental effects on organoleptic properties of *salchichón*.

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EFFECT OF BIOPRESERVATIVES AND LACTIC CULTURES ON SENSORIAL CHARACTERISTICS OF FERMENTED PORK SAUSAGES

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SUMMARY - In order to observe differences on sensorial characteristics in sausages we evaluated 210 fermented pork sausages elaborated with starter cultures and biopreservatives. Different batches of fermented sausages were fabricated: control, added with commercial starter cultures and added with commercial biopreservatives. Sensorial analysis was carried out for each sausage from the five batches after 30-days ripening by 12 trained panellists. During each testing session three samples were evaluated by each assessor for the follows attributes: colour, smell intensity, flavour intensity, hardness and general acceptability. No significant differences were appreciated between different lots ($p>0.05$).

Key Words: biopreservatives, starter cultures, fermented pork sausages, sensorial characteristics

INTRODUCTION

"Salchichón" is a typical spanish dry fermented pork sausage produced by the hurdles technology being implicated by the five following factors: nitrite and salt as preservatives decreased of redox potential (Eh), competitive lactic flora, and lowered pH and water activity (a_w). Usually this product has been made with a traditional process based on slow ripening process. Therefore the addition of cultures benefits only slightly if you compares with the traditional slow process. Although ripening process standardization is needed, the product quality and the trend towards shorter ripening times have obliged to use starter in manufacture on an industrial scale. Starter of lactic acid bacteria (LAB) produces the reduction of pH and gives place to the appearance of some metabolites with antimicrobial action (lactic acid, hydrogen peroxide and bacteriocines). Also, the addition of starter cultures tries to accelerate the proteolysis and lipolysis to intensify the development of the aroma and flavour in these meat products.

Occasionally the use of starter cultures can have undesirable improvements on sausage fermentation. Faster acid formation ranked very low and excessive acid formation, often associated with colour defects, sometimes also with gas formation, appears to be the main defect of fermented sausages (Lücke *et al.* 1990).

In last years biopreservatives has been used as an alternative to addition of starter. Biopreservation refers to the extension of storage life and enhanced safety of foods using the natural microflora and (or) their antibacterial products (Stiles, 1996). Biopreservatives has been used in fresh meat to extend shelf life of products and to enhance hygiene safety; keeping original sensory characteristics (Aymerich and Hugas, 1998). In fermented sausages the addition of biopreservatives containing metabolites of lactic acid bacteria allows to lead the ripening process and make more healthy and natural products (Mata *et al.,* 2001).

The aim of this study was compared the effect of the addition of biopreservatives and lactic cultures on sensorial characteristics of salchichón

MATERIAL AND METHODS

Five different batches of sausages were prepared, each with a different starter culture (batch D and E) and biopreservatives (batch B and C). Control is batch A (no bacteria added). Sausage production was carried out using the following recipe: 70% of pork meat, 30% back fat, 21 g/kg salt, 18 g/kg dextrose/glucose, 4.5 g/kg grain pepper, 2 g/kg phosphate, 2 g/kg nitrite salt and 1 g/kg other spice. The mix, previously minced in the cutter, was stuffed into a 60 mm-diameter artificial casing and subject to fermentation for 48 hours at 20º C and 95% relative humidity (RH). It was later transferred to a drying chamber at 12º C and 80 % RH for 28 days.

Different cultures and biopreservatives were added in the cutter during minced. Batch B was added with fermentation liquors of a culture of *Lactobacillus* sp. (Mata, 2000) and batch C with fermentation liquors of a culture of *Propionibacterium shermanii (Daeschel, 1989).* Then, batch D was added with a starter culture (*Lactobacillus sake*, *Staphylococcus xylosus* and *Staphylococcus carnosus*) in proportion 1:1:1 and batch E with a proportion 2:1:1.

Sensorial analysis was carried out for each sausage from the five batches after 30-days ripening by 12 trained panelists; selected and trained in accordance with the ISO 8586-1 standard (1993). During each testing session three samples were evaluated by each assessor for the follows attributes: smell intensity, colour, flavour intensity, hardness and general acceptability.

Statistical analysis of results was carried out using the software package SAS (2001) and variance analysis and mean comparison were determined.

RESULTS AND DISCUSSION

Table 1 shows the values of the sensorial attributes for the fermented pork sausages batches analyzed. As can be see, batch A sausages showed the higher scores for smell intensity, colour, flavour intensity and general acceptability. This sausages was made without biopreservatives or starter added. In other side, the sausages of batch E (with commercial starter) showed the lower scores for the same attributes and the higher score for hardness. In spite of this, significant differences were not detected (p>0.05). The other batches present similar scores between them.

Table 1. Variance and means comparison. Average values and standard deviations for sensorial parameters in the sausages batches at 30 days of ripening

Batch	Smell intensity	Colour	Flavour intensity	Hardness	General acceptability
A	6.54 ± 0.25	6.62 ± 0.26	6.52 ± 0.21	5.03 ± 0.43	6.56 ± 0.13
B	6.01 ± 0.20	6.20 ± 0.21	6.20 ± 0.32	4.84 ± 0.08	6.00 ± 0.12
C	6.22 ± 0.18	6.12 ± 0.45	6.26 ± 0.44	4.44 ± 0.67	5.87 ± 0.59
D	5.70 ± 0.47	6.17 ± 0.15	6.35 ± 0.40	5.20 ± 0.06	5.97 ± 0.26
E	5.68 ± 0.68	5.96 ± 0.14	6.21 ± 0.42	5.20 ± 0.29	5.86 ± 0.13
Probability (*P*<0.05)	NS*	NS	NS	NS	NS

***** NS: no significant differences

In a similar study Sanz *et al.* (1997) reported no differences in terms of colour, smell intensity and flavour between sausages with or without starter cultures, although other authors have described very high scores in colour of sausages added with cultures containing *Staphylococcus spp*. because of nitrate-reductase activity of this microorganism (Hartmann *et al.,* 1995; Flores and Toldrá, 1993). Also, Gonzalez-Fernandez *et al.* (1997) observed higher values for smell and flavour intensity in sausages with lactic cultures, although these sausages showed higher hardness. This is according with our results, sausages with starter showed highest scores in hardness in opposite of sausages with biopreservatives.

Finally, sausages with starter cultures obtained higher scores in general acceptability although no significant differences were reported between different batches (p>0.05). Similar results were obtained by Keneally *et al.* (1998) but other authors observed that sausages elaborated with starter showed higher acceptability (Gonzalez-Fernández *et al.*, 1997; Garriga *et al*., 1996).

From the result obtained, we can not be established that addition of starter cultures or biopreservatives in salchichón modifies significantly sensorial characteristics of product, although further studies are needed to evaluate the potential use of biopreservatives.

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Effect of mincing size and salt content in the quality of Painho de Portalegre – a Portuguese traditional sausage

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SUMMARY - Painho de Portalegre is a typical Portuguese sausage from Alentejo region. Its shape is cylindrical with a length usually between 25cm and 30cm and a diameter between 4cm and 6 cm. Two batches of Painho de Portalegre made with meat and fat from Alentejano pig breed were prepared in a small regional enterprise. One batch were prepared using meat and fat portions with 1,7 x 1,7cm (small portions) and another using portions with 5,5 x 2,5cm (large portions). Each batch (small portions and large portions) was divided in two: one with about 0,5% of NaCl in the end product and other with approximately 1% of NaCl. Final product obtained after 40 days of cure period was analysed trough microbiological, sensorial and rheological analysis. Microbiological and sensorial analysis didn't exhibit significant differences when comparing products with small and large portions. In rheological analysis, cohesiveness was significantly higher in products made with small portions, confirming a better liaison of meat and fat portions in those products. A general approach of sensorial evaluation results concluded that sausages with small portions exhibited a better classification than those made with large portions, although none of the sensorial parameters showed significant results from ANOVA.

Key words*:* Alentejano pig breed; Portuguese traditional sausage; quality; meat and fat size.

INTRODUCTION

The consumption of traditional Portuguese sausages was increased in the last years, mainly those that were made with fat and meat from Alentejano pig breed, an autochthonous Portuguese breed. Some of those food products were protected by Portuguese and European authorities, being commercialized under the Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI). The production increase of traditional sausage in Alentejo region causes evident benefits on regional economy. Producers of Alentejano pig breed have sell guarantee for factories and also for fresh meat consumption, on the other hand, the increase of the number of factories create new jobs and opportunities. So both aspects together are promoter of social and economical enrichment.

The goal of this work is to evaluate microbiological, sensorial and rheological characteristics of "Painho de Portalegre" made with two different portions size of meat and fat referred as small portions and large portions.

MATERIAL AND METHODS

Preparation of Painho de Portalegre and sampling

"Painho de Portalegre" is a traditional cured and smoked sausage, commercialized under the Protected Geographical Indication. Chilled chunks of pork lean and fat, from Alentejano pig breed, were grinded to obtain two batches mincing sizes: one of about 1,7cm x 1,7cm (small portions) and other with 5,5cm x 2,5cm (large portions). Each batch (small portions and large portions) was divided in two: one with approximately 0,25% of NaCl (corresponding a NaCl concentration about 0,5% in the end product, considering a water loss near 50%, among the process to obtain "Painho de Portalegre) and other with approximately 0,5% of NaCl. Each one of the four batches obtained were mixed and added of paprika paste, NaCl and garlic paste. After a holding period of 3 days at 0-2˚C, the mixture was stuffed in natural casings (rectal portion of pig intestine) and transferred to a traditional drying/smoking house (firewood burning inside) until an aw value of 0.88-0.85 was reached. Depending on the prevalent atmospheric conditions, this period varied between 30 to 40 days. For this study, after 40 days of the ripening process, tree samples were taken for each batch ([1)]0,5% NaCl small portions; [2)]1% NaCl small portions; [3)]0,5%NaCl large portions; [4)]1% NaCl large portions).

Microbiological analysis

Samples of 10 g were aseptically taken from the inner part of each sausage and homogenised for 3 minutes with Buffered Peptone Water (BPW) (Merck, Germany) at dilution 1:6 (w/v) in Stomaker Blender Seward 400 (London, UK). Serial decimal dilutions were made in BPW and then plated in duplicate for bacterial counts. Aerobic mesophilic bacteria were enumerated on Tryptone Glucose Extract Agar (Merck, Germany) after 48 h incubation at 30°C. Psychrotrophs were enumerated on Tryptone Glucose Extract Agar (Merck, Germany) after 10 days incubation at 6,5°C. Yeast numbers were determined on Yeast Extract Agar (Merck, Germany) after 5 days of incubation at 25°C. Aerobic mesophilic bacteria spores counts were determined on Tryptone Glucose Extract Agar (Merck, Germany) after 48 h incubation at 30°C. Inactivation of vegetative cells was made by immersion on water-bath during 10 minutes at 80°C. *Micrococcaceae* were enumerated on MSA (Manitol Salt Agar) (Oxoid,UK) and lactic acid bacteria on double-layer MRS agar (Man Rogosa and Sharpe, Oxoid, UK) after 72 h incubation at 30°C. *Enterobacteriaceae* counts were determined on VRBG (Violet Red Bile Glucose Agar, Oxoid, UK) after 48 h incubation at 37°C. *Enterococci* enumerations were made on Kanamicina aesculin Azide Agar (Oxoid, UK) during 48 h at 37°C. Clostridia sulfit-reducers spores were incubated on Sulfit Polimixin Sulfadiazine Agar (Merck, Germany) during 72 h at 44,5°C. Inactivation of vegetative cells was made by immersion on water-bath during 10 minutes at 80°C. Coliforms analysis was made on Brilliant Green Bile Lactose Broth (Merck, Germany) during 48 h at 30°C. For *Escherichia coli* analysis, from proof tubes where coliforms had grow, 0,01 ml were inoculated in proof tubes with Brilliant Green Bile Lactose Broth (Merck, Germany) and 0,01 ml were inoculated in proof tubes with Peptone Water (Merck, Germany). Temperature incubation was 44,5°C during 48 h.

Sensorial evaluation

The sensorial evaluation was conducing according to a descriptive quantitative method, with a scale from 0 to 100 and 12 trained panellists were used. The attributes considered were: color intensity, aroma intensity, tenderness, fibrousness, succulence, intensity of taste, undesirable taste, salt intensity, global evaluation.

Rheological analysis

The mechanical evaluation was performed using a Texture Analyser Stable. MicroSystem mod. TA-Hdi and respective software. The tests performed were a Texture Profile Analysis (TPA) with a compressed platen and a cutting test with a blade knife. The samples for the first test were cylindrical with 3,5cm of diameter and 3,5cm of height and were compressed twice to 10% of the initial height. For the cutting test samples were slices with 4mm of height and was measured the force (cutting strength) needed to cut 87,5% of the sample. In TPA considered parameters were: hardness, cohesiveness, springiness, gumminess and chewinness. For rheological tests 10 samples were used from each modality (small portions and large portions).

Data analysis

In order to analyze the results of microbiological study mean, maximum and minimum values were calculated. Rheological and sensorial results were analyzed trough multifactorial analysis of variance, considering factors “Portions size” (with 2 levels: small portions and large portions) and “salt concentration” (with 2 levels: 0,5% of NaCl and 1% of NaCl) and its interaction. Significant differences of means among batches were determined at a confidence level of 95%. STATISTICA software was used.

RESULTS AND DISCUSSION

The number of aerobic mesophilic bacteria didn't exhibit evident differences for the factors studied, portions size and salt concentration (tables 1 and 2)**,** however the sausages with higher salt concentration, 1% NaCl, had also higher values of aerobic mesophilic bacteria. These results allow us to suppose that the usual flora of these sausages as salt tolerance. Same interpretation can be done for the enumeration of yeasts. On the other hand, *Enterobacteriaceae* and *Enterococci* exists in higher level in samples obtained from sausages with lower salt concentration (0,5% NaCl). Considering that those groups of microorganisms are associated with hygienic conditions of processing can be achieved the benefit role of salt as inhibitor of microbiological grow.

Lactic acid bacteria, usually present in high level in traditional Portuguese sausages, exhibit the similar levels for different portions size and different salt concentration,. In spite of this, *Micrococcaceae* presented higher values in sausages produced with large portions and lower salt concentration, however generally these microorganisms grow in high salt concentration (Carrascosa-Santiago, 1989; González-Fernández *et al*., 1997; Santos *et al*., 1997; Hugas *et al*., 1998; Stahnke *et al*., 2002).

Aerobic mesophilic bacteria spores showed a higher number in sausages processed with larger portions of meat and fat.

Table 1 – Results of microbiological analysis in "Painho de Portalegre" produced with small portions and two different concentrations of salt (0,5% and 1% NaCl)

	0,5% NaCl			1% NaCl		
	Minimum	Mean	Maximum	Minimum	Mean	Maximum
Aerobic mesophilic bacteria (u.f.c.g^{-1})	1,00E+07	2,75E+07	4,60E+07	6,00E+07	8,53E+07	9,83E+07
Psychrotrophs (u.f.c.g^{-1})	6,25E+06	1,76E+07	3,00E+07	3,45E+07	7,02E+07	1,09E+08
Yeasts (u.f.c.g^{-1})	1,00E+02	4,00E+02	1,00E+03	3,50E+03	1,91E+04	2,97E+04
Micrococcaceae (u.f.c.g^{-1})	1,06E+06	3,90E+06	8,30E+06	7,40E+04	2,45E+05	4,60E+05
Lactic acid bacteria (u.f.c.g^{-1})	2,47E+08	2,88E+08	3,17E+08	1,29E+08	2,59E+08	4,15E+08
Aerobic mesophilic bactéria spores (nº spores g^{-1})	1,10E+03	1,28E+03	1,45E+03	3,40E+03	9,50E+03	2,10E+04
Enterobacteriaceae (u.f.c.g^{-1})	5,00E+02	1,63E+03	3,20E+03	6,00E+02	8,00E+02	9,00E+02
Enterococci (u.f.c.g^{-1})	4,40E+05	7,17E+05	9,00E+05	2,80E+05	4,57E+05	7,90E+05

Table 2 - Results of microbiological analysis in "Painho de Portalegre" produced with large portions and two different concentrations of salt (0,5% and 1% NaCl)

	0,5% NaCl			1% NaCl		
	Minimum	Mean	Maximum	Minimum	Mean	Maximum
Aerobic mesophilic bacteria(u.f.c.g^{-1})	3,90E+07	6,68E+07	9,15E+07	1,75E+07	8,08E+07	1,27E+08
Psychrotrophs (u.f.c.g^{-1})	2,90E+07	3,98E+07	4,90E+07	3,85E+06	8,37E+06	1,63E+07
Yeasts (u.f.c.g^{-1})	1,50E+03	9,33E+03	1,45E+04	4,98E+04	1,07E+05	1,46E+05
Micrococcaceae (u.f.c.g^{-1})	5,60E+06	1,11E+07	1,53E+07	3,00E+05	4,60E+06	1,08E+07
Lactic acid bacteria (u.f.c.g^{-1})	2,33E+08	2,51E+08	2,60E+08	2,10E+08	3,47E+08	5,90E+08
Aerobic mesophilic bactéria spores (nº spores g^{-1})	2,50E+04	4,27E+04	6,90E+04	4,60E+04	6,50E+04	9,50E+04
Enterobacteriaceae (u.f.c.g^{-1})	9,25E+03	1,42E+04	2,00E+04	2,00E+02	3,83E+03	9,10E+03
Enterococci (u.f.c.g^{-1})	3,20E+06	5,27E+06	7,60E+06	9,80E+05	2,89E+06	5,40E+06

All the other microorganisms analyzed, such as *Clostridium* sulfite-reducers spores, coliforms and *Escherichia coli* (Table 3 and 4) didn't exhibit any differences for the different modalities of sausages processing.

Table 3 – Results of ***Clostridium* sulfite-reducers spores**, **coliforms and *Escherichia coli* analysis** for "Painho de Portalegre" produced with 0,5% and 1% of NaCl, for sausages produced with small portions.

NaCl Concentration (%)	**Microorganism Classification**	***Clostrídium* sulfite-reducers spores** (nº of samples)	**Coliforms** (nº of samples)	***Escherichia coli*** (nº of samples)
	class 1	3		
	class 2			
0,5	class 3			1
	class 4		2	2
	class 5		1	
	class 1	3		
	class 2			
1	class 3			
	class 4		1	3
	class 5		2	

Legend:
Class 1: < 1 bacteria/g
Class 2: ≥ 1 bacteria/g <10 bacteria/g
Class 3: ≥ 10 bacteria/g <100 bacteria/g
Class 4: ≥ 100 bacteria/g < 1000 bacteria/g
Class 5: ≥ 1000 bacteria/g < 10000 bacteria/g

Table 4 – Results of ***Clostridium* sulfite-reducers spores**, **coliforms and *Escherichia coli*** analysis for "Painho de Portalegre" produced with 0,5% and 1% of NaCl, for sausages produced with large portions.

NaCl Concentration (%)	**Microorganism Classification**	***Clostrídium* sulfite-reducers spores** (nº of samples)	**Coliforms** (nº of samples)	***Escherichia coli*** (nº of samples)
	class 1	3		
	class 2			
0,5	class 3		2	3
	class 4		1	
	class 1	3		
	class 2			
1	class 3			
	class 4		2	3
	class 5		1	

Legend:
Class 1: < 1 bacteria/g
Class 2 : ≥ 1 bacteria/g <10 bacteria/g
Class 3 : ≥ 10 bacteria/g <100 bacteria/g
Class 4 : ≥ 100 bacteria/g < 1000 bacteria/g
Class 5 : ≥ 1000 bacteria/g < 10000 bacteria/g

The variance analysis of the data from sensorial evaluation revealed that there aren't any significant differences for any attribute. However, some tendencies can be noticed (Table 5). Products obtained with small portions exhibit more intense aroma, and the fibrousness was strong for the sausages obtained with large portions. The color of samples with lower level of salt was more intense but products with a higher level of salt presented superior values for tenderness (confirmed by rheological tests), succulence and intensity of flavor, for the last attribute referred sausages with 1% of salt show 5 points more then the others produced with 0,5% of salt. Higher values for succulence and flavor in the sausages with 1% of salt can be explained by the increment of saliva which fact cause a better perception of flavor. However, the panel only noticed the differences of salt content in the sausages made with small portions: 56 points for sausages with 0,5% NaCl and 64 points for those with 1% NaCl. The global appreciation in sensorial analysis evidenced a preference for the products processed with small portions.

Table 5 – **Sensorial analysis** - Means and standard deviation for two factors “portions size” and “salt concentration”

Portions size	NaCl (%)	Color intensity	Aroma intensity	Tenderness	Fibrousness	Succulence	Intensity of taste	Undesirable taste	Salt intensity	Global evaluation
Small	**0,5**	75,6±9,3	69,4±12,3	65,0±16,6	31,4±22,1	66,75±9,7	67,6±10,6	5,2± 8,4	56,0±8,9	71,2±11,2
portions	**1**	61,8±18,2	65,0±8,8	74,6±14,5	29,8 ±19,9	70,0±10,0	72,2±5,3	8,0±13,0	64,0±11,4	69,0±6,5
Large	**0,5**	78,6±11,8	63,4±15,6	69,6±12,3	35,0±19,4	68,8±8,8	65,2±5,8	4,0±8,9	55,4±9,8	64,4±9,3
portions	**1**	69,6±10,0	63,0±21,7	76,6±11,1	43,2±29,7	75,0±12,3	70,6±9,5	7,0±12,4	55,2±10,8	66,0±16,7

Related with rheological evaluation (Table 6), cohesiveness was the parameter that presented significant differences (p<0,01) for the factor size of portions (0,74 for small portions and 0,68 for large portions) (Table 7**)**. However, other parameters such as springiness and chewiness, obtained also higher values in the sausages produced with small portions (Table 7**)**. These results can be due to a higher specific surface in the mince made with small portions. It causes a higher extraction of soluble protein and consequently a stronger liaison in mince. On the other hand, the cutting strength was significantly lower (p<0,05) in the sausages produced with small portions.

Table 6 **– Rheological analysis** **-** Analysis of variance for two factors “Portions size” and “Salt concentration”

	Portions size (PS)			**Salt concentration (SC)**			**Interaction (PS x SC)**		
	F Value	p Value	Significance	F Value	p Value	Significance	F Value	p Value	Significance
Hardness	0,075	0,7867	NS	0,112	0,7413	NS	0,035	0,8541	NS
Cohesiveness	9,368	0,0062	**	0,002	0,9633	NS	0,031	0,8616	NS
Springiness	2,487	0,1305	NS	0,159	0,6941	NS	0,211	0,6512	NS
Guminess	0,008	0,9276	NS	0,084	0,7744	NS	0,015	0,9023	NS
Chewiness	0,178	0,6776	NS	0,015	0,9048	NS	0,0001	0,9932	NS
Cutting strength	7,131	0,0147	*	0,023	0,8801	NS	0,042	0,8392	NS

Legend: NS – not significant, p≥0,05; * p<0,05; ** p<0,01

Table 7 **– Rheological analysis** - Means and standard deviation for two factors “Portions size” and “Salt concentration”

Portions size	**NaCl** (%)	**Hardness** (N)	**Cohesiveness**	**Springiness**	**Guminess** (N)	**Chewiness** (N)	**Cutting strength** (N)
Small	**0,5**	1006,0±825,6	0,744±0,037 a	0,842±0,068	761,3±650,8	648,7±571,6	2321,7±524,6 a
portions	**1**	970,0±345,2	0,740±0,049 a	0,840 ±0,091	730,5±294,0	628,0±282,6	2376,0±680,0a
Large	**0,5**	1117,8±624,4	0,682±0,040 b	0,761±0,096	767,2±420,9	580,2±293,9	3608,7±1729,5 b
portions	**1**	991,3±471,8	0,684±0,060 b	0,795±0,127	690,5±367,1	562,3±338,0	3532,0±1271,4 b

Different letters in the same column represent significantly different means.

CONCLUSIONS

A general appreciation of the results indicates that the microbiological population didn't change for the factors portions size and salt concentration. However *Enterobacteriaceae* and *Enterococci* were at a large number in sausages produced with 0,5% NaCl.

On the other hand, *Micrococcaceae* and yeasts were higher for sausages produced with large portions.

Sensorial analysis didn't exhibit any significant differences, but the panel noticed more intense aroma in sausages produced with small portions and more intense color in products processed with 0,5% NaCl, but sausages with salt content of 1% obtained a better classification for tenderness, succulence and taste intensity. The panel preferred the sausages processed with small portions, which preference was revealed in the global evaluation, and only was detected the difference amount of salt in those products.

Rheological analysis confirmed a stronger liaison of the mince made with small portions that exhibit simultaneously higher cohesiveness, showed in TPA, and better tenderness, showed by a smaller cutting strength.

This approach allows us to think that is possible to produce high quality sausages with lower salt content, using the traditional technological process with adequate hygienic conditions.

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MUSCLE FIBER CHARACTERISTICS OF BLACK SLAVONIAN PIG – AUTOCHTHONOUS CROATIAN BREED

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SUMMARY - The histochemical and histomorphological characteristics (diameter and proportion of red slow-twitch oxidative (SO), white fast-twitch glycolytic (FG) and intermediate fast-twitch oxidative glycolytic (FOG) fibre types) of *M. longissimus dorsi* (MLD) in Black Slavonian Pig (BS, n=10) in comparison to modern crossbred (Large Weight x Swedish Landrace sired with Duroc; CB, n=8) pigs were analysed. BS pigs (153.8 average live weight/18 mounths of age) and CB pigs (182.1 kg average live weight/18 months of age) were reared and fattened in outdoor production system under the same feeding conditions. The FG fibers of large diameter dominate in MLD of both BS and CB pigs (69 and 75.8 % of total cross sectional area). The proportion of FG fiber was lower (69.10 vs. 76.5 %, P<0.01) and their diameter was smaller (68.5 vs. 72.10 µm, P<0.05) in BS than CB pigs. Also, BS pigs showed higher proportion of SO (11.7 vs. 8.4 %, P<0.01) and FOG (19.6 vs. 15.8 %, P<0.01) fibers than CB pigs. The diameter of SO (64.0 vs. 67.5 µm, P<0.05) but not FOG fiber was smaller in BS than CB pigs.

Key Words: Black Slavonian Pig, muscle fiber characteristics, meat quality

INTRODUCTION

The Black Slavonian pig (BS) breed is an autochthonous Croatian breed originated in the second half of the 19 century in East Croatia. It is characterized by dark colour, robust constitutions, and slower growth rate with higher adiposity and reduced lean deposition in comparison to modern white pigs. Traditionally, BS pig production is under extensive management in outdoor production system, and feeding is based on utilization of natural resources of pasture and oak (*Quercus robur L.*) woodland with supplement of a small amount of corn seed or some other cereal (~ 0.15-0.50 kg per head daily). The short preslaughter fattening period with corn based diet is a common practice. Typical slaughter weight for BS pigs is between 160 and 180 kg. In the past, its meat was used for local dried meat specialties, like Slavonian Kulen and ham. Recently, these products are producing from the meat of modern crossbred pigs (CB) of variable quality attributes. There are only few studies comparing meat quality of BS and modern CB pigs under extensive management. Histochemical properties of a muscle, such as fibre type composition, fibre diameter, oxidative and glycolytic capacities, and glycogen and lipid contents, as factors that have been found to influence meat quality in BS pigs were not investigated. The objective of this study was to investigate histochemical and histomorphological properties and meat quality parameters of BS pigs in comparison to modern CB pigs under extensive management.

MATERIAL AND METHODS

The study was performed on 19 pigs of the local Black Slavonian (BS) breed, 10 castrates and 9 females and 8 modern crossbred (CB) pigs (Duroc x (Swedish Landrace x Large White), 4 castrates and 4 females. All of the animals were reared in outdoor production system and maintained under same management and feeding programme. The examined pigs were reared on pasture utilizing nature resources with the addition about of 0.5 kg of concentrate daily per pig. A kg of concentrate had 12.5 MJ ME and 13.0 % crude protein (CP). At the slaughter average live weight was 153.8 kg and 182.1 kg in both BS and CB pigs, respectively. The pigs were 18 mounts of age. Both, BS and CB were free from C1843T mutation on RYR1 locus (Fuji *et al*., 1991). Pigs are slaughter in the same abattoir located 10 km from farm after exposure to same preslaughter treatments.

Shortly after slaughter (30-45 min) muscle samples for histochemical analysis from 10 BS and 8 CB pigs were taken. Samples were taken from middle portions of m. longissimus dorsi (MLD) at the last rib level. These samples were frozen in liquid nitrogen and stored at -80 C until analysis. Muscle pH was measured using Testo portable pH meter at 45 minutes and 24 h post-mortem on longissimus muscle at last rib level. On the same place muscle colour (CIE *L**, *a** and *b**) were measured by Minolta 410.

The histochemical profile was determined by staining 8 µm transverse serial sections for SDH activity and with myosin ATPase method at different pH. Diameter and proportions of muscle fibre types were analysed on two different picies of each muscle samples and more than 200 muscle fibres per sample were analysed. The muscle fibre diameter was measured on cross-sections of individual muscle fibers using a Nikon microscope and a Leitz microscale with 10 µm divisions.

Statistical analyses were preformed using the SAS software version 8.1 (SAS, 1999). The differences in muscle fiber characteristic between BS and CB pigs were studied using the analysis of covariance including carcass weight as a covariate (PROC GLM) The relationship between histochemical profile and meat quality parameters in both BS and CB pigs were tested using Pearson´s correlation test (PROC CORR).

RESULTS AND DISCUSSION

Red slow-twitch oxidative (SO), white fast-twitch glycolytic (FG) and intermediate fast-twitch oxidative glycolytic (FOG) fibres were found in the samples of *M. longissimus dorsi* (MLD) in both, BS and CB pigs. Proportions of each muscle fibre type and their diameter in BS and CB pigs are shown in Table 1.

Table 1. Breed effect on histochemical characteristics and meat quality traits in *m. longissimus dorsi*

Fibre type	Breed		Significance
	Black Slavonian LSM (SE)*	Crossbreed LSM (SE)*	
Percentage			
SO	11.70 (0.42)	8.40 (0.47)	0.01
FOG	19.60 (0.63)	15.80 (0.70)	0.01
FG	69.10 (0.66)	76.50 (0.74)	0.01
Diameter (µm)			
SO	64.00 (1.53)	67.50 (1.71)	0.05
FOG	54.89 (1.32)	56.30 (1.41)	N.S.
FG	68.50 (1.18)	72.10 (0.99)	0.05
Meat quality traits			
pH_i	6.44 (0.05)	6.54 (0.06)	N.S
pH_u	5.77 (0.04)	5.69 (0.06)	0.05
L	48.11 (1.22)	50.15 (1.63)	N.S
a	9.27 (0.46)	12.60 (0.71)	0.01
b	3.04 (0.49)	6.22 (0.83)	0.01

* Least square means and standard errors

The white fibres (FG) of large diameter predominate in MLD of both BS and CB pigs (69 % and 75.8 %) and they show a strong activity of standard (S ATP) and alkaline stable adenosine triphosphatase (AL ATP) and a weak activity of succinate dehydrogenase (SDH) and acid stable adenosine triphosphatase (AC ATP). On the other hand, the red fibres (SO) are the lowest numerous fiber type in MLD and they show a strong activity of SDH and AC ATP, and a weak activity of S ATP and AL ATP. The proportion of FOG fibres were intermediate and their diameter were smaller in comparison to FG and SO fiber types. Also, they are characterized with a strong activity of S ATP and AL ATP, a weak activity of AC ATP and a moderate activity of SDH. Significant differences among breed were found for all the histochemical characteristics studied, except for diameter of FOG fiber type. BS longissimus muscle had a higher proportion of SO and FOG fibers and lower proportion of

FG fiber than CB pigs (P<0.01). In addition, the diameter of SO and FG fiber was smaller in BS than in CB pigs (P<0.05). There was no difference in diameter of FOG fibres, which was the smallest fibre type in both breeds.

In the past, several studies on longissimus muscle, have reported differences in muscle fibre characteristics between wild/unimproved and modern selected pigs (Rahelic and Puac, 1980; Ruusunen and Poulanne, 1997; Serra *et al*., 1998; Brocks *et al*., 2000; Oksbjerg *et al*., 2000). Results of these study are in agreement with our results and showed that selection toward high lean meat content and growth rate caused changes of proportion of muscle fiber types in the direction to increase the proportion of FG and decrease the proportion of SO and FOG fibre type. In addition, the fibre diameter in highly selected pigs increased in comparison to that of traditionally breeds at the same age.

As a result of intensive selection changes in meat quality is often to occur. Selections to increase carcass lean content have resulted in increase glycolitic and reduce the oxidative capacity of the muscle (higher proportion of FG fibres of large diameter, lower proportion of SO fiber and increase their diameter). Therefore, fibre type composition cold have an influence on pre- and post-mortem muscle metabolism and subsequently effect on meat quality (Maltin *et al*., 1997; Klont *et al*., 1998; Karlsson *et al*., 1999). In this study, meat quality traits in both, BS and CB pigs were within expected range for normal meat (Kaufmann *et al*., 1992; Sellier i Monin 1994; Joo *et al*., 1999). Differences between breed were observed for ultimate pH, redness and yellowness of MLD. In BS longissimus muscle were observed higher ultimate pH (P<0.05) and lower redness-*a* (P<0.01) and yellowness-*b* (P<0.01) than in CB pigs. Correlations between fibre type percentage and meat quality traits of MLD in both, BS and CB pigs are shown in table 2.

Table 2. Correlations between fibre type percentage and meat quality traits of MLD within BS (upper diagnal) and CB (lower diagonal) pig.

	% SO	% FOG	% FG	pH_i	pH_u	L
% SO		-0.16	-0.62*	0.20	-0.09	0.41
% FOG	-0.42		-0.82*	0.21	-0.19	-0.44
% FG	-0.25	-0.70*		-0.30	0.22	0.17
pH_i	0.34	-0.13	-0.14		-0.25	-0.29
pH_u	-0.48	-0.28	0,65*	0.19		0.41
L	0.39	-0.28	-0,26	-0.66*	-0.55	

Correlations with an asterisk are significant at P<0.05.

No consistent relationship between an each fiber type and a meat quality trait was found across breed. Significant and positive correlation was found only between percentage of FG fibre type and pH_u value in CB but not in BS pigs. Some previous studies (Maltin *et al*., 1998; Serra *et al*., 1998; Chang *et al*., 2003) found correlations between some histochemical characteristics and meat quality traits. Chang *et al*. (2003) were found significantly positive correlation between both, slow MyHC I and MyHC 2x fibres and ultimate meat pH in the *psoas* but not in MLD muscle. In addition, they were found negative correlation between colour saturation and amount of fast glycolityc MyHC 2b fibres in MLD of Duroc, and *psoas* of Large White (LW) and Tamworth. The same authors suggest that increasing amount of slow oxidative or/and oxidative-glycolytic fibres could have a beneficial effect on meat quality and the presence of wide variation in the biochemical and histomorphological properties of the same fibre type between breeds what could be supported with results of this study.

CONCLUSIONS

FG fibers of large diameter predominate in MLD of both BS and CB pigs. Therefore, MLD could be described as a fast twitch or white muscle. BS pigs have lower proportion of FG fibres and higher proportion of SO and FOG fibre in comparison to CB pigs, indicating that the longissimus muscle in BS had a higher oxidative capacity in comparison to CB. The diameter of both FG and SO fibres was lower in BS than in modern CB pigs.

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INFLUENCE OF SEASONING TECHNOLOGY ON CHARACTERISTICS OF LONG RIPENED SALAMI PREPARED WITH LIMITED QUANTITY OF SALT

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SUMMARY - The salami are considered traditional products, because they are prepared with methods used before the industrial development. The acknowledgement of origin denominations (IGP, DOP) confirms this hypothesis. The study of used technologies, in the various centuries and in the several regions, allows to evidence some variations between the traditional techniques and those used in handicraft and industrial systems. These changes, substantially, are linked with the necessity to increase the salami consistency and to reduce the preparation time acting on the modalities of mixtures preparation, on the ingredients choice (salt and/ or of sugars increase) and on the ripened modalities, with medium temperatures increase used, especially during the first part of the process. However exist some productions, generally to familiar level, that travel over origin again more traditional techniques; the salami, therefore prepared, have got organoleptic characteristics (less acids, slice less cohesive, less salt) appreciated from a consumers part even if this productions do not show always the same qualitative level and involve important refuse of productions also. The use of techniques that travel oven again traditional methods, based on the control of the mixtures modalities and on the use of temperatures and ripened time more traditional (a short heating of the fresh product: 1 or 2 days in according to the diameter of the product, first ripened to refrigeration temperature for the necessary time to reduce the Aw (inferior values to 0,94)) allow the preparation of the traditional products characterised from limited salt amount (<2,0%) and limited other ingredients amount (sugar lack).

Key words: Salami, seasoning technology, long ripening, salt

INTRODUCTION

Evolution of productive techniques from handicraft to industrial

The salami are between the most diffuse traditional products meat; some types are recognized like IGP or DOP also from the European Union (es. Salamini italiani alla cacciatora, salame Brianza, salame Cremona, etc).The study of the modalities of preparation adopted in the various centuries and the several regions, also lacking some important information, allows to evidence some differences between the traditional techniques and those most recent ones.

When we talk about traditional techniques should identify the period referred to; a reference to the very distant period (Roman, medieval) is probably misleading because the dietary habits and lifestyles were too different, so it is better to take as a reference techniques developed in the first half of the twentieth century, indicating these as traditional, and compare with those developed in 50-60 years' at the transition of many products to industrial and handicraft.

These innovations have affected the manner of preparation of the mixtures, the choice of ingredients and additives (increase in salt and / or sugar, use of nitrite alone or in combination with nitrate) and the techniques of seasoning, with the average increase temperatures, especially in the early stages.

The amount of salt used in traditional preparations vary from region to region and from area to area depending on, among other things, environmental conditions and / or eating habits, in the province of Parma recipes can be found with quantities of between 2.3 -2.5% (essentially areas of hill and mountain average) and 1.8-2.2% (many areas of the plain), these values are different from those of the first recipes industrial average higher (2.5 2.8% for the Felino salami and values even more high for other Italian productions: 2.8-3.3%).

Salami prepared with limited quantity of salt (<2%)

Because salt is also an essential ingredient for the control of microbial multiplication (including harmful germs) a reduction must take place in a technology that considers all aspects in order to avoid incidents of various kinds: proliferation of harmful germs, High acidification, poor sealing of the slice, etc..

If you are preparing products not strongly acidified and quantity of additives and ingredients very limited the control of microbial flora depends on two factors physical and chemical physical: temperature and Aw, since the reduction of Aw is a phenomenon slow the temperature must be maintained at lower values than those of normal industrial production. This necessarily implies an increase in the maturing time that must be considered in the "design" of the product.

On this basis has been tested a technique that has as a key step reduction of Aw, up to a level equal to or less than 0.94, in conditions of refrigeration in order to obtain salami less acidified and with low amounts of salt as in traditional recipes of Padana plain's.

There were several preliminary tests conducted, not mentioned in this statement, which made it possible to identify as satisfactory a technique based on resting of meat for 2-3 days at temperatures < 1 ° C, on preparation of meat mixture, on resting of salami mixture for 2-3 days at temperatures <3 ° C, on stuffing salami mixture in natural casing, on resting salami for 2-4 days at temperatures <4 ° C, on drying them for a day at room temperature <20 ° C, on the cold ripening salami for 4 weeks at temperature <5 ° C and on ripening salami for 3-6 months at temperature <15 ° C

MATERIAL AND METHODS

Composition mixture

In this note are reported data confirmatory test conducted on type Felino salami stuffed in natural pig casing: average weight of 1.8 kg prepared with meat and fat pig blacks (Cinta Senese) and white (Large White) with salt (1.8%), pepper (0.08%), potassium nitrate (150ppm) garlic (0.01%) and wine (0.5%). The chemical characteristics of the physical and chemical mixtures are presented in Table 1

Table 1. Composition mixtures salami white (Large White) and black pigs (Cinta Senese)

	Large White	Cinta Senese
Moisture (%)	57,19	53,13
Proteine	17,46	17,6
Fats	22,88	26,68
Salt (NaCl %)	1.75	1,79
Aw	0.978	0,976
pH	5.80	5,78

Conditions for ripening

The conditions thermo-hygrometric maintained in the various phases are reported in table. 2.

Table 2. Terms thermo-hygrometric the various stages of preparation

phase	days	Medium temperature	Medium Relative Humidity
Resting of fresh meat	3	-1,29	62,14
Resting of salami Mixture	3	1,54	79,20
Resting of stuffed Salami	3	1,07	83,49
Drying	1	18,48	57,61
First cold ripening	19	1,50	85,46
Second cold ripening	22	4,01	86,09
Ripening	219	13,69	72,54

Drying

This technique is similar in many respects to traditional, still taken in domestic production, the adoption of a period, even though very short (1 day), drying temperature close to that environment is intended to provide a degree activation energy for micro-organisms responsible for the maturation while for traditional products also had the task to facilitate the drying of the casing.

The drying at room temperature was limited to a single day to reduce to acceptable levels the possibility of proliferation of some seeds of alteration (es enterobacteriaceae), characterized, at Aw values and pH of the product, from reduced duplication time.

Ripening

Compared with industrial techniques, and which are the subject of the majority of technical scientific publications, the most innovative is the cold ripening after drying. To avoid at this stage dangerous “drying rim”, dehydration must be particularly slow due to the slower diffusion of water at low temperature, and consequently the weight loss is limited.
The methods of dehydration are manifold; the graph Figure 1 lists the Relative Moisture variation in time for the test conducted at the pilot plant of the Stazione Sperimentale of Parma. By transferring this technique in different installation, industrial or craft, the modes of dehydration, and thus RH variation over time may be different while it is appropriate that temperatures are taken of the same order of magnitude.

The choice of time of the cold ripening should be function of various parameters including the likelihood of multiplication of seeds of alteration during the next necessary step in maturing at room temperature, which is not maintained at high values. By choosing this temperature must be used forecasting systems of microbial multiplication (es. total coliforms) consider that the population (maximum tolerable and predictable) germs of alteration, maturing temperature, and pH Aw salami.

In this case the choice of time of cold ripening has been carried out and also above all to obtain satisfactory functional characteristics such good consistency of the product, good grip, colour, weight loss adequate (> = 20%). These conditions (time of cold ripening no less than 4 weeks, Aw after cold ripening = <0.94, ripening temperature between 13 and 14 ° C) ensure control of the multiplication of pathogenic germs that are inactivated rather slowly, but always within the time frame end of warm ripening (3-5 months).

Ripening Time

Of course the ripening times are longer and thus techniques similar to the one described are eligible only for products with specified characteristics.

RESULTS AND DISCUSSION

Evolution of microbial population

The long maturation allow the inactivation of harmful germs present in fresh even in high number (in production pilot with heavily contaminated meat total coliforms have risen from 10 ^ 6 fresh in less than 10 ufc / g in ripened products). The evolution of the microbial flora, as regards other germs, does not differ in its broad outlines, the same way as other types of salami.

A study of the strains may be directed to the identification of particular favourites selections and / or stop determined by low temperature of the dough, salami and those already undergoing treatment drying.In technological conditions indicated the weight loss are high and salami at the end of seasoning may submit a consistency very hard, if this negative element is the use of traditional pig meat (ex. Cinta Senese) gives better products. (Table 3).

Table 3 losses salami weight of pigs white and blacks at different times ripening

	Large White		Cinta Senese	
	%	Ds	%	Ds
resting (66 days*)	24,29	2,68	22,47	2,68
first ripening (127days*)	37,74	2,27	32,21	2,12
second ripening (183 days*)	43,54	2,08	36,90	1,20

L (*) Days from start of drying salami

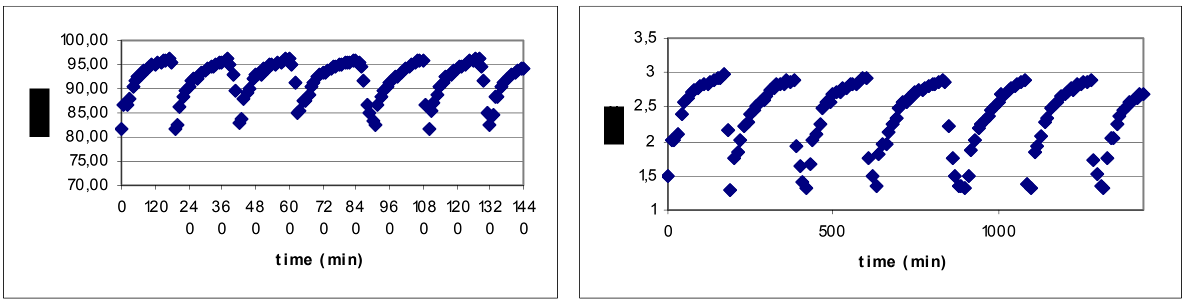
Black pigs

The use of black pigs can be a source of some incidents linked to the characteristics of the tissues of these animals. The majority in-saturation adipose tissue (increased presence of linoleic acid as initially reported Tab.4) was one of the causes of poor outcome for some productions. It is therefore appropriate preparation techniques for the two different types of reducing meat for salami Cinta, the mixing time of mixture and drying temperatures that, although high, may promote oxidation and the partial melting fat forming an impermeable layer close to the gut.

Table 4. fatty acid composition (%) fat (bacon) used for tests with white and Cinta Senese pigs

	Breeds pigs		
Fatty acids	**Large White**	**Cinta senese 1**	**Cinta senese 2**
C10	0,09	0,06	0,03
C12	0,09	0,07	0,04
C14:0	1,43	1,57	0,88
C16:0	25,28	24,02	18,00
C16:1	2,55	3,38	1,97
C17	0,29	0,27	0,24
C17:1	0,32	0,35	0,34
C18:0	12,17	10,01	8,10
C18:1	47,49	47,76	51,88
C18:2	8,49	10,66	14,64
C18:3 n-3	0,51	0,47	0,63
C20	0,14	0,10	0,14
C20:1	0,68	0,70	1,57
C20:2	0,32	0,41	1,14
C20:3n-6	0,04	0,04	0,10
C20:4	0,09	0,04	0,09
C20:5	0,06	0,08	0,25

Fig 1 Behaviour of RU% and Temperature in cold ripening



CARCASS AND MEAT QUALITY TRAITS OF PIGS WITH DIFFERENT BLOOD FRACTIONS OF "MORA ROMAGNOLA" BREED, REARED OUTDOORS

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SUMMARY – Nowadays, the Mora Romagnola (MR) is a small numbered autochthonous Italian pig breed, and, thus, is often crossed with genetically improved animals, including commercial hybrids, to get subjects which are reared outdoors for the production of fresh meat and traditional seasoned salami. This research aims to get preliminary information on both carcass and meat quality traits of either purebred MR, or PICxMR (MR50), or (PICxMR)xMR (MR75), reared outdoors in an organic pig farm on Reggio Emilia Apennines, in Italy. Examined carcases (4 MR, 5 MR50, and 7 MR75), though showing high and extremely variable weights (MR 152.2 kg, MR50 245.3 kg, MR75 214.4 kg), produced very valuable lean cuts contents (MR 60.1%, MR50 53.3%, MR75 56.0%) and fair adipose cuts contents (MR 32.0%, MR50 41,2%, MR75 37,2%). On average, *Longissimus dorsi* muscle (LD) contained 69.1% water, 22.4% protein, and 6.8% fat, with no difference among the genetic types. Colour and pH values of thigh and LD muscles at 24h p.m. showed optimal values, suitable even for PDO production.

Key words: *Autochthonous pig, Mora Romagnola, Carcass quality, Meat quality*

INTRODUCTION

Autochthonous pig breeds, kept in several areas mainly in family-farm systems and devoted to the production of salami and fresh meat, have been for a long time the focus of Italian pig rearing. The raising of people's purchasing power and the consequent increase of meat demand, together with the improved rearing techniques, led to the gradual replacement of the local breeds, rustic but at low productivity, with the so-called "improved breeds", coming from other Countries and showing high productive and reproductive performance. Many local breeds have now disappeared, with a consequent loss of genetic variability, and those survived to extinction counts very few animals. Anyway, in the last years consumers have become more concerned about questions such as ethical forms of animal productions, animal welfare, respect and growing sensitivity towards the environment, and request for more information about the whole quality of the dietary products (Mayoral *et al.*, 1999). As a consequence, a part of the pig productive sector has been pushed towards the re-discover of more natural rearing systems, aiming to preserve the remaining local breeds, and linking them to the territory in order to give high quality products to the consumers.

Local breeds are usually reared by organic systems and, because of the low numbers of animals, they are often introduced in crossing schemes with improved breeds, to obtain products different from those derived from the latter.

Mora Romagnola is one of the breeds reared in Emilia Romagna Region. The number of animals of this breed had fallen, at the beginning of '90, to very few pure-bred subjects, reared in one farm and strongly blood relative (Giannone, 2002). A recovery plan designed by WWF Italia and Torino University (Fortina *et al.*, 2001) allowed to gain a number of 450 animals, distributed in 45 farms and registered to the Registro Anagrafico of the Associazione Nazionale Allevatori Suini (Italian Pig Breeders Association). A proper number of animals should be the first step to provide this breed with an economical position, linked to the creation of a small production niche. Like the largest part of the still existing local breeds, the Mora Romagnola is crossed with improved animals, such as Large White, Landrace or sometimes commercial hybrids. The cross-breeds subjects are used for fresh meat and seasoned salami production, which are mainly commercialized in the production area. Due to the rearing system, which is almost exclusively outdoors and in organic farm, the products have a high added value.The interest gained by the Mora’s products makes important the study of their quality, to provide consumers with correct information which enable to distinguish them from the industrial ones normally offered by market. The aim of this first step of the study is to evaluate the carcass and meat quality characteristics of pigs derived from Mora Romagnola breed.

MATERIAL AND METHODS

Mora Romagnola pure-breed pigs (4 animals) and cross-breeds with 50 and 75% of Mora blood, obtained from the cross between pure-breed boars and PIC hybrid sows (5 and 7 animals, respectively), were used. The pigs were reared in outdoor by organic farming and slaughtered on different days.The weight of each carcass and the fat thickness in three different anatomical locations (gluteus medius, last rib and maximum thickness at the shoulder) were measured before carcass cutting. At cutting, 4 hrs after carcass chilling at 2-4°C, the weight of all lean (thigh, shoulder, neck, loin), fat (backfat, belly, jowl, and perirenal fat) and bone cuts (head, tail, foots), were recorded. The pH and the colour were measured on the longissimus dorsi (LD), from the 4^{th}/ 5^{th} - dorsal vertebra. A sample, composed by all soft tissues and by bone basis, was then cut from the LD, including the 4^{th} the $5^{th}$and the 6^{th} dorsal vertebrae, to evaluate the incidence of the different tissues (muscle, fat, bone and connective). pH and colour measurements were repeated 24 hrs post mortem, and samples of *LD* were collected for the analysis of chemical composition (AOAC, 1990). All thighs were weighed, and the pH and colour of *semimembranosus* (SM) and *biceps femoris* (BF) muscles were measured. When the thighs were trimmed, 24 hrs after cutting, the lean and fat components of rejected parts were separated, and weighed; moreover, the measurements of pH and colour were repeated on the same muscles. Data were subjected to analysis of variance according to the genetic type; backfat thickness and cuts weight were co-varied by carcass weight.

RESULTS AND DISCUSSION

Table 1 reports carcass weight and backfat thickness measured in three different points on the splitting line of the carcass. Carcass average weight was 208.5 kg, but the variability, due to the heterogeneous sample, was very high. Carcass weight was very different among the three genetic types: pure-breed pigs produced lighter weights, whilst the heavier remarks came from 50% Mora Romagnola pigs. Genetic type isn’t anyway the only responsible for these differences, since pigs lived outdoor with free-range reproduction, which might cause different ages at slaughter. Live weight at slaughtering was around 255 kg, common for this type of production but much higher than the usual weight of pigs destined to the PDO systems, about 160-165 kg (Lo Fiego *et al.*, 2000).

Since slaughtering weight was very different among the three genetic types, backfat thickness was co-varied on the first. This analysis could not relieve any difference for the measures taken at gluteus medius muscle and at the last rib, whilst the thickness at the shoulder was significantly higher in pigs with 75% of Mora Romagnola blood. Backfat thickness observed in this trial is anyway much higher in comparison with the measures commonly taken nowadays in Italian heavy pigs (Lo Fiego *et al.*, 2005; Santoro *et al.*, 2006), but is very similar to the measures taken in the 130-140 kg carcasses of improved pigs in the ‘70ies (Ceci and Guizzardi, 1978).

Table 1. Carcass traits according to the genetic type

	Overall average (n.16)	Estimated average for Mora blood percentage			(13 d.f.)	EMS (13 d.f.)
		100%	50%	75%	75%	
Carcass weight kg	208.5 ± 56.5	152.2	245.3	214.4	*	43.76
Backfat thickness mm (1)						
gluteum gluteus medius	45.1 ± 10.4	45.2	46.2	44.3	n.s.	8.11
last rib	44.5 ± 11.9	46.9	43.5	43.8	n.s.	7.47
shoulder (maximum)	63.7 ± 14.7	64.2	60.0	66.1	*	9.77

*= P< .05; n.s. = not significant; 1) Co-varied on carcass average weight (12 d.f.)

Tables 2 and 3 report cuts weight and incidence. The average weight of the different cuts was very high. No significant difference was found for the carcass co-varied values of the cuts among the three genetic types, with the exception of shoulder weight, which was significantly higher in pure-breed pigs. This may be explained if one reminds that local breeds, when not influenced by the effect of the improved breeds, maintain a different proportion between front and back quarter.

Genetic selection applied to the PDO systems pigs favoured a greater development of back quarter, for the needs of seasoned ham production (Nanni Costa *et al.*, 1993); on the opposite, the so-called traditional-rustic pigs showed, before the empiric selection applied on them, a much greater development of the front quarter, and these difference are still probably present.

Table 2. Carcass cuts weight (kg) according to the genetic type (data co-varied on carcass average weight)

	Overall average (n.16)	Estimated average for Mora blood percentage			EMS (12 d.f.)
		100%	50%	75%	
Loin	14.38 ± 4.16	13.63	13.97	15.09	1.28
Thigh at 4hrs p.m.	21.91 ± 5.83	21.62	21.36	22.48	2.09
Thigh at 24hrs p.m.	21.16 ± 5.88	20.66	21.29	21.37	1.52
Trimmed thigh	16.88 ± 4.55	16.18	16.22	17.66	1.47
Shoulder*	13.06 ± 3.52	16.16	11.73	12.22	1.15
Neck with bone	8.88 ± 2.43	9.61	8.37	8.84	0.84
Backfat	21.83 ± 9.27	17.99	24.60	22.03	3.13
Belly	8.58 ± 2.38	8.38	8.89	8.42	1.96
Jowl	4.23 ± 1.48	4.13	4.11	4.38	1.01
Perirenal fat	5.06 ± 2.64	5.76	5.57	4.60	1.83
Head	4.92 ± 1.34	4.79	5.06	4.89	0.87
Foots	1.63 ± 0.35	1.51	1.59	1.60	0.21

*= genetic type significant for P<.01

From the table 3, which reports the incidence of the carcass cuts, we can see that, although slaughtered at very high weights, these pigs provide a very good percentage of lean cuts: the values of the pure-breed pigs are similar to the remarks of the common Italian heavy pigs (Santoro *et al.*, 2006).

The average percentage of lean cuts was 60.1% for Mora pigs, showing a significant higher incidence for the shoulder and, higher but not significant, for the neck. The average incidence of fat cuts was 37.15%, and the values was significantly higher for 50% Mora pigs: the difference is mainly due to backfat, which was 23.5% *vs* 14 and 21% of the pure-breed and of the 75% Mora pigs, respectively. We can notice on the whole that more than the half of the cuts is constituted by lean cuts, whilst the other cuts (fat and other) have a all-in incidence of about 44% (37% and 7%, respectively).

Table 3. Cuts incidence on carcass (%)

	Overall average (n.16)	Estimated average for Mora blood percentage			Stat. Signif	EMS (13 d.f.)
		100%	50%	75%		
Loin	13.76 ± 1.24	13.25	13.40	14.31	n.s.	1.21
Thigh at 4hrs p.m.	20.72 ± 1.33	20.80	20.55	20.82	n.s.	1.45
Trimmed thigh	16.62 ± 1.57	16.77	15.64	17.14	n.s.	1.55
Shoulder	12.71 ± 2.06	15.65	11.68	11.77	**	1.18
Neck with bone	8.60 ± 1.00	9.55	8.07	8.45	n.s.	0.91
Total Lean cuts	56.21 ± 3.95	60.13	53.33	56.02	*	3.24
Backfat	19.97 ± 4.51	13.96	23.49	20.89	**	2.74
Belly	8.45 ± 1.99	9.46	8.35	7.95	n.s.	2.11
Jowl	4.05 ± 0.89	4.10	3.77	4.23	n.s.	0.93
Perirenal fat	4.67 ± 1.57	4.52	5.56	4.12	n.s.	1.57
Total Fat cuts	37.15 ± 4.30	32.04	41.17	37.18	**	2.77
Other cuts	7.38 ± 1.73	8.76	7.74	6.67	n.s.	1.52

**= P<.01;*= P< .05; n.s. = not significant

Table 4 reports the incidence of the different tissues on the loin sample cut (including the 4th, the 5th and the 6th dorsal vertebrae) and the chemical composition of the LD. The sample cut was composed by 58% muscle, the half of which was LD (27.6 %); bone was 18.7%, and fat 19.3%. No significant difference was found among the three examined genetic types: nevertheless, 75% Mora pigs showed a higher incidence of muscle (60% *vs* 56.5% of the pure-breed and 55.4% of 50% Mora pigs). LD (table 4) was composed on the average of 69.1% water, 22.4% protein and 6.8% fat, without any difference among the genetic types. Data show a good protein content of the meat of these animals, although slaughtered at very high weight. Similar values were found by other authors in pigs of Cinta Senese breed slaughtered at 151 to 162 kg live weight (Sirtori *et al.*, 2007). Lipid level was higher in comparison with both those reported by the same authors (5.7% on the average) and with those found in PDO systems heavy pigs (3.1%; Lo Fiego *et al.*, 2000). About the sensory traits of the meat, the higher lipid content provides a more pronounced taste which may be appreciated by consumers.

Table 4. Incidence of the different tissues on loin sample cut (4th, 5th and 6th dorsal vertebrae) and chemical composition of the *longissimus dorsi* muscle [(1)]

	Overall average (n.16)	Estimated average for Mora blood percentage			EMS (13 d.f.)
		100%	50%	75%	
Tissue incidence on sample cut (%)					
Bone	18.72 ± 4.21	16.72	20.98	18.26	3.48
Fat	19.33 ± 4.35	20.87	20.40	17.69	4.13
L. dorsi muscle	27.57 ± 5.54	30.36	24.60	28.09	5.59
Other lean tissue	30.19 ± 5.02	26.09	30.78	32.10	4.39
Chemical composition of *l. dorsi* muscle (%)					
Water	69.07 ± 2.68	69.95	67.58	69.62	2.72
Protein	22.41 ± 1.22	21.92	22.22	22.69	0.95
Fat	6.76 ± 3.75	7.50	8.67	5.26	3.47

[(1)] No differences among genetic type (P>.05)

Table 5 reports pH and colour values of muscles LD, BF and SM measured at 4 and 24hrs *post mortem*. The pH, without any effect of Mora blood percentage, showed values considered optimal also in PDO ham sector. These values are slightly higher than those normally measured in commercial pigs, but clearly lower than the values reported for this breed by other researchers (Fortina *et al.*, 2005). The pH values recorded at carcass cutting were already quite low, thus indicating a quite quick glycolysis, but this didn't negatively affected muscle colour: L* values were in fact normal and not influenced by genetic types, although meat tended to be slightly darker for pure-breed LD, and for 50% Mora thighs.

Table 5. pH and color values of *l. dorsi* (LD), *biceps femoris* (BF) and *semimembranosus* (SM) muscles at carcass cutting (4 hrs) and after 24 hrs of chilling[(1)]

	Overall average (n.16)	Estimated average for Mora blood percentage			EMS (13 d.f.)
		100%	50%	75%	
pH LD 4hrs	5.60 ± 0.15	5.74	5.60	5.52	0.13
pH LD 24hrs	5.64 ± 0.19	5.79	5.61	5.57	0.15
pH BF 4hrs	5.64 ± 0.19	5.64	5.68	5.61	0.21
pH BF 24hrs	5.63 ± 0.16	5.72	5.66	5.55	0.6
pH SM 4hrs	5.63 ± 0.25	5.65	5.75	5.53	0.26
pH SM 24hrs	5.65 ± 0.20	5.72	5.67	5.59	0.20
Colour (L* value)					
LD 4hrs	42.93 ± 6.47	37.78	41.26	47.16	5.65
LD 24hrs	44.57 ± 4.74	43.42	44.72	45.02	5.28
BF 4hrs	36.36 ± 4.36	35.72	34.08	38.54	4.27
BF 24hrs	39.44 ± 4.82	39.85	38.65	39.78	5.27
SM 4hrs	37.54 ± 4.03	37.95	36.35	38.16	4.24
SM 24hrs	38.67 ± 3.15	37.71	36.69	40.63	2.72

[(1)] No differences among genetic type (P>.05)

CONCLUSIONS

An increasing sensitivity of consumers for environment problems and the re- discovering of old traditional products lead today some productive sectors, although marginal, towards the re-introduction of breeds whose numbers cause a risk of extinction.

This trend allowed the slow down of some autochthonous breeds extinction, and contemporarily drew researchers' attention towards the problem of bio-diversity protection, very important for genetic variability. Furthermore, one should pay attention to the growing demand about the qualitative and nutritional traits of the products derived from these "new" productive systems.

The results of this first research about the Mora Romagnola breed provide a picture of a productive niche. Although slaughtered at high live weight, pure-breed animals and cross-breeds with genetically improved animals gave carcasses providing amounts of lean cuts very similar to those of the improved breeds destined to the heavy pigs production in the PDO areas. Meat pH and colour were in the optimal range also for PDO products; meat showed a very good protein level, and its fat content was higher than that of improved breeds.

Although modern consumers ask leaner and leaner foods, the relatively high fat content of Mora meat may be appreciated, since this improves its taste.

Pure-breed animals and their cross-breeds didn't show, in our research, relevant differences for the evaluated parameters. This result may be partly attributed to the high heterogeneity and low number of examined animals.

The main aim of our research was to give a first picture of a local productive system, which is recent and very peculiar: this should give knowledge for experimental plans including the effects of several factors on carcass, meat and products quality on larger numbers of animals.

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MEAT QUALITY OF WILD BOARS, PIGS AND CROSSBREED REARED IN BONDAGE

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SUMMARY – In order to study the meat quality of wild boars, pigs and crossbreeds (F_1 and F_2), 16 sample of Longissimus dorsi have been analysed, 4 of which came from wild boars, 4 from pigs, 4 from F1 crossbreed (wild boar x pig) and 4 from F_2 crossbreed [wild boar x (wild boar x pig)]; all the animals have been reared in pigsty and slaughtered at 9 mouths of age. Into the results we can note, even if with different levels of statistic significance ($P \leq 0,05$ e/o $P \leq 0,01$), that the wild boar's longissimus dorsi shows lower "L", "a", "b" indices, higher tenderness, greater cooking reduction, and as the F_1 samples, higher percentage of water, protein and lower level of fat. Moreover the wild boar meat fat, even if with different levels of statistic significance ($P \leq 0,05$ e/o $P \leq 0,01$), is characterized by a higher percentage of $C_{18:2CLA}$, of $C_{20:2\omega6,}$ of $C_{18:1\omega9}$, by a greater ratio of unsaturated/saturated and ω_6/ω_3, and by lower levels of $C_{6:0}$ and of $C_{17:0}$.

Key words: Meat quality, wild boars, bondage, unsaturated fatty acids.

INTRODUCTION

The quanti-qualitative aspects of meat depend on several factors such as genotype, feeding, sex, age of the animal, rearing system, slaughter conditions, as well as on carcass dissection and domestic cooking (Giorgetti e Poli, 1990). In the last years the changes in socio-economic conditions of the consumers in developed countries have led to a change in their feeding habits. More and more attentive to the relation nutrition/health, the modern consumer requires foods, and therefore also meats, wholesome and of good quality, poor in fat and in saturated fatty acids such as the $C_{12:0}$, the $C_{14:0}$ and perhaps C_{16}, that can increase the plasma level of cholesterol (Hegsted *et al*., 1965; Grundy 1986; Bonamone e Grundy, 1988), but rich in unsaturated and polyunsaturated fatty acids, promoters of the lowering of LDL cholesterol (Mattson e Grundy, 1985), which the risks of heart disorders are connected to. Such risks can be reduced thanks to the consumption of polyunsaturated fatty acids of the ω-3 series (Connor, 1997), the role of which in the prevention of thrombosis, atherosclerosis and cardio-circulatory disorders has been reported also by Barsottelli e Berra (1994), by Hornstra *et al.* (1975); by Vergoesen *et al.* (1980); by Carlier *et al.* (1991) and by Kinsella *et al*. (1990).

If the idea of wholesomeness is to be connected to a reduced human interventions in the productive cycle and the utilization of eco-friendly technologies, such as open door rearing or the utilization of autochthonous animal genotypes, the idea of quality is surely connected to the product chemical, physical and organoleptic traits, which can satisfy the needs, evident or not, of the consumer. Wild genotypes can be well identified with such ideas since their meat offers an image of both wholesomeness and quality.

In the last 50 years, the total meat per-capita consumption in Italy has passed from 20.5 kg in 1955 to 91.1 kg in 2002 (ISMEA, 2003) even if in the last decade the average consumption of the meats from some species has registered sometimes a contraction and even a stagnation depending on sanitary emergencies (BSE, Blue Tongue, etc.). On the contrary , swine meat consumption did not register such negative trends and even exceeded the beef one. In 2005 Italy registered a consumption of swine meat of 23 kg approx. per-capita (Assiteca New, 2006); while in Europe the per-capita consumption rose up to 42.9 kg, even though in the last years a reduction of 3-4 percentage points was registered.

The rearing of wild genotypes, together with the autochthonous ones, is to be more and more assumable in those inner territories at marginal productivity that, after the second postwar have been expanding in our country, partly due to the flight of peasants towards more industrialized areas, partly because of the concentration of farming on the plains and coasts, surely more fertile.

Among the wild genotypes, the wild boar (*Sus Scropha L.*), together with hybrids with the domestic pig, seems to be qualified to be reared in those areas and under all rearing conditions (extensive, semi-extensive, intensive raring), due to acceptable productive performances (Marsico *et al*., 1990 a,b; Fabbri e Bergonzini, 1980; Centoducati *et al*., 1991; Vicenti *et al*., 1991; Marsico *et al*., 1993; Vicenti *et al*., 1994; Marsico *et al*., 1997 a, b) , but with high quality (Marsico *et al*., 1994; Marsico *et al*., 1997; Laudadio *et al*., 1997; Marsico *et a*l., 1997; Marsico *et al*., 2000; Marsico *et al*., 2001; Marsico *et al*., 2002; Marsico *et al*., 2003 a,b,c; Dimatteo *et al*., 2003; Marsico *et al*., 2004; Dimatteo *et al.*, 2006).

The literature we consulted on pig rearing offers useful information on the quanti-qualitative aspects of its productions (Barbari e Ferrari, 1997; Cole, 1987; Ferrarini *et a*l., 1992; Franci *et al*., 2001; Just, 1986; Lo Fiego *et al*., 1990; Manari, 1991; Mayoral *et al*., 1999; Mordenti, 1986; Pugliese *et al*., 2002; Russo, 1975; Sather *et al*., 1997). On the contrary, the comparison among the different quanti-qualitative traits of the meat of wild boars, pigs and hybrids does not present enough informations. For this reason, the present research paper aims to assess the carcass composition and the quality traits of the meat of wild boars, pigs and their hybrids F_1 (wild boar x pig) and F_2 [wild boar x (wild boar x pig)].

MATERIAL AND METHODS

Sixteen animals were divided per genotype in 4 groups of 4 animals each. The animals were reared in a pigsty and given a complete feed, the chemical and percent composition of which is reported in Tables 1 and 2.

All the animals were slaughtered at the age of 9 months. After slaughter, the carcasses were stored at 4°C for 24 hours. Afterwards, the right half side was dissected into cuts according to the local habits but approaching as much as possible to ASPA recommendations (1996).

On a sample of raw Longissimus dorsi (LD), the colorimetric indexes “L”, “a” and “b” (Hunter Lab system), pH (1 and 2 after 24 h of storage), the cooking losses (in oven at 180°C up to a core temperature of 75°C), the content in drip loss, hardness (kgf/cm) and the cutting force by Warner Blazer shear force test were measured (WBS).

On a part of raw LD the chemical composition (ASPA, 1980) has been measured and from the extracted fat (Folk et al., 1957) the fatty acid percent distribution has been determined using a gas-chromatography system with a 60 m capillary column in silica glass with stationary phase in cyanopryl film at 100%, after methylation. To identify the single fatty acids, we referred to the retention periods in comparison with the palmitic acid (C16:0) and when in doubt to well-known standards.

All the collected data were subjected to the analysis of variance using a GLM procedure and the significance between means evaluated using Student’s “t” test (SAS, 1996)

RESULTS AND DISCUSSION

Some physical parameters of the considered muscle, even if with a different level of statistical significance ($P \leq 0,01$ e $P \leq 0,05$), are influenced by animal genotype (Table 3). In particular, as concerns colour, pig meat presents a higher lightness than wild boars (53,81 vs 44,38; $P \leq 0,01$) and, together with F_1, than F_2 and wild boars (53,81 e 50,57 vs 48,53 e 44,38; $P \leq 0,05$). The index of yellow “b” resulted significantly ($P \leq 0,01$) higher in hybrids F_1 and F_2 than wild boars and pigs (13,09 e 13,47 vs 8,18 e 10,14). PH_1 detected showed higher values in F_2 animals than the other groups (6,91 vs 6,41, 6,47 e 5,95; $P \leq 0,01$). The muscle of F_2 and wild boars in comparison with the pigs presented higher hardness (3,05 Kgf/cmq e 3,27 Kgf/cmq vs 2,02 Kgf/cmq; $P \leq 0,01$) and a greater resistance for F_1, wild boars and pigs than F_2 (2,40 cm, 2,18 cm e 2,43 cm vs 1,49 cm; $P \leq 0,01$). Moreover, wild boar meat presented a higher cooking loss than F_2 and pigs (14,64% vs 10,11% e 9,93%; P≤0,01) and registered a higher percentage of drip loss than the other genotypes (1,50% vs 0,68%, 1,12% e 0,97%; P≤0,01).

As concerns the chemical composition (Table 4), higher percentages of moisture for F_1 and wild boars than pigs (73,65% e 73,41% vs 71,60%; P≤0,01) and of the same than F_2 (73,65% e 73,41% vs 72,22%; P≤0,05). Wild boars presented, on the contrary, a higher incidence of proteins than F_2 (22,50% vs 21,51%; P≤0,01) and together with F_1, than the other genotypes (22,50% e 22,24% vs 21,51% e 21,89%; P≤0,05).The fat percentage was higher for F_2 and pigs than the other two groups (3,60% e 3,33% vs 2,15% e 2,00%; P≤0,01), while ashes were greater in F_2 and wild boars than F_1 and pigs(1,47% e 1,30% vs 1,27% e 0,88%; P≤0,01), and, even with a lower significance (P≤0,05) for F_2 than wild boars (1,47% vs 1,30%).

As concerns the fatty acid composition (Table 5), among the saturated fats $C_{6:0}$ was more present in the muscle of pigs than F_1 (0,15% vs 0,01%; P≤0,05), as well as $C_{17:0}$ than the other groups (0,48% vs 0,21%, 0,23% e 0,28%; P≤0,01). $C_{15:0}$ was higher in wild boars and pigs than both hybrid groups (0,10% e 0,10% vs 0,04% e 0,08%; P≤0,01), whereas $C_{18:0}$ significantly (P≤0,05) higher in pigs than F_1 (14,60% vs 11,59%). Generally, for the unsaturated series, even if with different levels of statistical significance (P≤0,01 e/o P≤0,05), percent variations for certain fatty acids, depending on animal genotype, are observed. In particular, it is noticed a higher percentage of $C_{16:1}$ for F_1, F_2 and wild boars than pigs (3,11%, 3,15% e 3,00% vs 0,08%), whereas wild boars presented a higher (P≤0,01) incidence of $C_{17:1}$ than the other groups (0,40% vs 0,18%, 0,28% e 0,20%), of $C_{18:1\omega9}$ (P≤0,05) together with F_2 and than F_1 (41,73% e 41,48% vs 37,49%), of $C_{18:1\omega7}$ (P≤0,05) than F_2 (4,23% vs 2,63%), of C20:1ω9 (P≤0,01) together with F_1 and in comparison with the other two groups (0,38% e 0,58% vs 0,08% e 0,18%) of $C_{18:2CLA}$ (P≤0,01) than F_1 and pigs (0,33% vs 0,01% e 0,01%), of $C_{20:4\omega3}$ (0,10% vs 0,01%, 0,01% e 0,01%; P≤0,01) and together with F_2, of $C_{20:5\omega3}$ (0,35% e 0,48% vs 0,01% e 0,08%; P≤0,01) than the remaining groups. $C_{18:3\omega3}$ was higher (P≤0,01) in F_1 and F_2 than the other groups (0,68% e 0,40% vs 0,18% e 0,05%), $C_{20:2\omega6}$ in F_2, wild boars and pigs than F_1 (0,83%, 0,80% e 0,80% vs 0,40%; P≤0,01), $C_{20:3\omega6}$ (P≤0,01) in pigs than the other groups (0,48% vs 0,17%, 0,08% e 0,10%), $C_{20:4\omega6}$ (P≤0,05), in F_1 than wild boars (0,69% vs 0,13%); at last, $C_{21:5\omega3}$ (0,08% vs 0,01%, 0,01% e 0,01%), of $C_{22:\omega9}$ (0,10% vs 0,03%, 0,01% e 0,01%) and of $C_{22:5\omega6}$ (0,10% vs 0,01%, 0,03% e 0,01%) in F_2 are higher (P≤0,01) than the other groups. On considering the total of fatty acids per series, the monounsaturated showed a higher percent (P≤0,05) in wild boars than F_1 and pigs (49,75% vs 44,42% e 44,48%), ω_3 (P≤0,01) in F_1, F_2 and wild boars than pigs (1,03%, 0,74% e 0,65% vs 0,16%). Moreover, the ratio ω_6/ ω_3 was higher (P≤0,05) for pigs than the other groups (18,67 vs 17,37, 10,74, e 14,65).

CONCLUSIONS

The research has evidenced that the genotype has significantly influenced meat quality. In particular, the wild boars presented a less bright and a softer colour meat, with intermediate acidity between F2 and pigs, more hard and resistant, with a higher content in free water and a higher cooking loss. Moreover, their meats resulted not only more proteic than the other genotypes, but also richer in minerals and with lower fat, in particular compared with the domestic pig. The fatty acid composition of wild boar meat, even presenting a higher incidence of some saturated fatty acids, is surely richer in monounsaturated , polyunsaturated and ω_3 particularly in comparison with pigs.

According to this, we may assert that wild boars and their hybrids present meats of higher quality for better both physical and chemical and acidic traits, in other words a high quality food products.

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Table 1: Chemical composition of feed (%)

	D. M.
Moisture	-------
Protein	13.96
Fat	6.01
Ash	9,11
Crude Fibre	5,63
Indeterminate	25,35
NDF	25,80
ADF	8,47
ADL	4,82
AIA	0,85
M.E. (kcal/kg)	3.372,20

Table 2: Feed composition (%)

	%
Maize	58,70
Oats	7,00
Barley	10,55
Soya meal	11,00
Grape skin	10,00
Salt & Integrators	2,75

Table 3: Physical parameters of meats (LD)

	Genotypes				ESD
Num. Samples	4	4	4	4	
	F_1	F_2	WB	P	(12 = DF)
L	50,57 ab	48,53 bc	44,38 Bc	53,81Aa	3,418
A	14,65	8,23	6,15	4,51	8,559
B	13,09 A	13,47 A	8,18 B	10,14 B	1,352
pH1	6,41 B	6,91 A	6,47 B	5,95 C	0,164
pH2	5,85	5,37	5,89	5,35	0,715
W.B.S. raw:					
Hardness (Kgf/cmq)	2,69	3,05 A	3,27 A	2,02 B	0,467
Resistance (cm)	2,40 Aa	1,49 B	2,18 Ab	2,43 Aa	0,118
W.B.S. cooked:					
Hardness (Kgf/cmq)	2,87	3,51	3,34	4,42	1,189
Resistance (cm)	1,84	2,17	1,76	1,85	0,714
Water loss (%)	11,72	10,11 B	14,64 A	9,93 B	1,904
Drip loss (%)	0,68 bC	1,12 B	1,50 A	0,97 aC	0,144

A, B, C: P<0,01; a, b: P<0.05

Table 4: Chemical composition (%) – raw LD

	Genotypes				ESD
Num. Samples	4	4	4	4	
	F_1	F_2	WB	P	(12 = DF)
Moisture	73.65 Aa	72.22 b	73.41 Aa	71.60 B	0.690
Protein	22.24 a	21.51 Bb	22.50 Aa	21.89 b	0391
Fat	2.15 B	3.60 A	2.00 B	3.33 A	0.467
Ash	1.27 B	1.47 Aa	1.30 Ab	0.88 C	0.084
Indeterminate	0.69 B	1.20 B	0.79 B	2.30 A	0.425

A, B, C: P<0,01; a, b: P<0.05

Table 5: Intramuscular fatty acids composition % (raw LD)

	Genotypes				ESD
Num. Samples	4	4	4	4	
	F_1	F_2	WB	P	(12 = DF)
Saturated series					
C6:0	0,01 b	0,10	0,05	0,15 a	0,082
C8:0	0,01	0,03	0,08	0,05	0,061
C10:0	0,14	0,13	0,15	0,13	0,050
C12:0	0,09	0,10	0,10	0,10	0,008
C14:0	1,38	1,48	1,48	1,40	0,167
C15:0	0,04 B	0,08	0,10 A	0,10 A	0,029
C16:0	25,21	25,10	25,08	25,65	1,502
C16:r	0,01	0,01	0,01	0,01	0,000
C17:0	0,21 B	0,23 B	0,28 B	0,48 A	0,076
C18:0	11,59 b	13,00	11,95	14,60 a	1,924
C20:0	0,13	0,20	0,13	0,10	0,077
C22:0	0,01	0,01	0,01	0,03	0,025
Unsaturated series					
C16:1	3,11 A	3,15 A	3,00 A	0,08 B	0,567
C17:1	0,18 Bb	0,28 aB	0,40 A	0,20 B	0,053
C18:1ω9	37,49 b	41,48 a	41,73 a	40,85	2,325
C18:1ω7	3,01	2,63 b	4,23 a	3,18	0,895
C 18:2ω6	11,17	9,08	11,13	7,93	2,618
C18:3ω3	0,68 Aa	0,40 Ab	0,18 Bc	0,05 B	0,126
C20:1ω9	0,58 Aa	0,08 B	0,38 Ab	0,18 Bc	0,095
C18:2CLA	0,01 B	0,10	0,33 A	0,01 B	0,095
C 20:2ω6	0,83 A	0,40 B	0,80 A	0,80 A	0,082
C20:3ω6	0,17 B	0,08 B	0,10 B	0,48 A	0,093
C20:4ω6	0,69 a	0,43	0,13 b	0,18	0,341
C20:4ω3	0,01 B	0,01 B	0,10 A	0,01 B	0,003
C20:5ω3	0,01 B	0,48 A	0,35 A	0,08 B	0,084
C21:5ω3	0,01 B	0,08 A	0,01 B	0,01 B	0,025
C22:1ω9	0,03 B	0,10 A	0,01 B	0,01 B	0,014
C22:5ω3	0,08	0,08	0,03	0,03	0,048
C22:5ω6	0,01 B	0,10 A	0,03 B	0,01 B	0,025
C 24:1ω9	0,01	0,04	0,01	0,03	0,049
total saturated	38,78	40,43	39,70	42,45	2,745
Total unsaturated	59.33	57.58	59,70	57,71	2,157
Monounsaturated	44,42 b	47,70	49,75 a	44,48 b	2,583
Polyunsaturated	13,17	11,63	12,74	9,95	3,053
ω6	12,43	10,60	12,58	9,30	3,015
ω3	1,03 Aa	0,74 Ab	0,65 Ab	0,16 B	0,182
ω6/ω3	17,37 b	10,74 b	14,65 b	18,67 a	63,923
Unsaturated/saturated	1,51	1,47	1,51	1,35	0,160
AI	0,54	0,53	0,55	0,52	0,050
TI	1,25	1,23	1,44	1,22	0,142
saturated/polyunsaturated	3,39	3,50	3,40	4,02	0,826
PCL/PCE	1,35	1,35	1,29	1,31	0,170

A, B: P<0,01; a, b, c: P<0.05

CHEMICAL, SENSORIAL, RHEOLOGICAL AND COLORIMETRIC CHARACTERISTICS OF "CAPICODDHO AZZE ANCA", A CURED HIND LEG OF PORK *CAPICOLLO* PRODUCED IN THE GREEK-CALABRIAN AREA.

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SUMMARY – "*Capicoddho Azze Anca*" – a cured hind leg of pork *capicollo* – is a cold cut typical of the ancient Greek area of Calabria (Italy). It is still produced in a homemade and traditional manner using the muscles of the thigh and natural flavorings. The physico-chemical, colorimetrical and sensorial characteristics of two *capicolli*, taken from normal businesses in production, have been examined, at 4 and 7 months of maturation (±15days), for each one of 3 companies. The chemical composition at 4 months, manifested average percentage values of 59.85, 26.31, 8.18 and 5.66, respectively for humidity, proteins, lipids and ashes, with 1.92% collagen and 4.19% salt. At 7 months, average percentage values of 50.38, 29.30, 9.43 and 10.89 were obtained respectively for humidity, proteins, lipids and ashes, with 1.82% collagen and 5.98% salt. Moreover, the *capicolli* at 4 months were slightly lighter in colour (L* 42.17 *vs* 39.78) and more tender (WBS 4.72 *vs* 5.44). Sensorial tests tended to show small differences.

Key words: Calabrian local product, *capicollo* quality, *capicollo* physico-chemical characteristics, *capicollo* sensorial characteristics

INTRODUCTION

The territory of the Grecanic area is located in the most southern part of the Province of Reggio Calabria (Italy), at the extreme tip of the Italian peninsula, along the valley of the Amendolea river and the Siderone and San Pasquale torrents. It has always been the cradle of the "hellenophonic" Calabrian linguistic minority and custodian of ancient and unchanged popular traditions which have the flavour of the cultural splendours of *Magna Graecia* (Rohlfs, 1933; Norwich, 1974; Ficarra, 1995; Cudia, 1999). In this area, in which pig breeding is very widespread and has deep roots, a variety of characteristic cured cold cut meats are produced, prepared even today according to "sacred" artisan methods and using recipes handed down over generations with the passion and the pleasure of those who leave to their descendants a piece of their history and of their culture.

The "*Capicoddho Azze Anca*" is one of these cured cold cuts, produced using the muscles of the hind leg and natural flavourings, among which, in particular there are wild fennel seed and red chilli pepper, which are characteristic of Calabria.

In the context of policies aimed at the rediscovery and valorisation of characteristic products which are being manifested in all the regions of Italy, and considering the fact that this cured cold cut is highly renowned *in loco*, but literally unknown outside its area of production, we feel that it certainly deserves to enjoy greater prominence. In this experimental work, we have sought to study its chemical, rheological, colorimetric and sensorial characteristics with a view to establishing an initial qualitative platform on which subsequent improving interventions can be based and which would be usefully indicative for the productive chain in this sector. In the production of the *capicollo* "*Azze Anca*" the thigh muscles of Large White Italiana" X "Nero Calabrese" half breeds are used. From these, three portions are obtained. The first, which is usually of larger dimension, is composed of the *semitendinosus* and *longus vastus* muscles. The second is formed largely from the *semimembranosus,* and the third, which is more round, from the *vastus medialis, vastus lateralis* and *rectus femori*.

MATERIAL AND METHODS

For each of three companies, indicated with the letters F, R, and T, representative of the production territory, two hind leg *capicolli* were taken from normal businesses in production. Each sample had been conserved in a vacuum pack at a temperature of 2-4° C before the analysis was carried out. The first, at 1Kg±100g, was composed of *semimembranosus* muscle and was matured to four months (±15days); the second, at 1,5Kg±100g, was composed of *semitendinosus* and *longus vastus* muscles and was matured to seven months (±15days). For both, in triple form, the following analyses were carried out: chemical (humidity, proteins, lipids, ash, collagen and salt - FoodScan Meat Analyzer Foss); colorimetric (C.I.E. L*a*b* - Spectral Scanner DV) and rheological (cutting resistance - WBS - Instron 5542).

Sensorial properties (odour, taste and savouriness) were determined using a randomly chosen jury of 20 evaluators, using a verbal hedonic scale with 6 judgement points for odour and savouriness and 9 for taste.

RESULTS AND DISCUSSION

Chemical analysis in the *capicolli* at four months of maturation (Fig. 1) gave an average humidity value of 59.85%±0.36, with a maximum of 60.21% for company T and a minimum of 59.48% for company R, manifesting maturation conditions apparently highly similar. The situation was *vice versa* for those at seven months (Fig. 2). The average percentage for humidity was 50.38±5.92, with a maximum value of 55.75% for company F and a minimum of 44.04% for company T, thus revealing, for these *capicolli,* that conditions of maturation would appear to be less similar among the three companies.

As far as proteins, lipids and ashes are concerned, the following average percentages were found respectively, at four months: 26.31±1.20, 8.18±1.05 and 5.66±0.18. Collagen was, on average, at 1.92%±0.09 and the presence of salt at 4.19%±0.04. At seven months of maturation, the following average percentage values were found: 29.30±3.20, 9.43±1.91 and 10.89±1.29, respectively for proteins, lipids and ashes, with collagen at 1.82%±0.26 and salt at 5.98%±0.05. On the whole, the results relative to the chemical composition of *Capicoddho Azze Anca* manifested in both maturation periods, a dietary-nutritional physiognomy which can be likened to the best cured cold cuts of the same typology (*culatello*). In particular, the results regarding the protein content are very interesting. Here we refer to the low level of lipids and, not least, the quantity of salt which is maintained at medium to low levels with regard to the standard for cured cold cuts of this kind.

As far as physical characteristics are concerned, the comparison between the two periods of maturation shows that the *capicolli* at four months were on average more tender (WBS 4.72±0.88 *vs* 5.44±0.31) (Fig. 3), slightly lighter in colour (L* 42.17±2.94 *vs* 39.78±1.80) with a level of intensity of red (a* 12.19±1.94 *vs* 12.42±3.41) and yellow (b* 7.02±3.13 *vs* 7.68±0.59) which could effectively be superimposed on those matured to seven months (Figs 4 and 5). Comparison among the companies, *vice versa*, has shown, by and large, appreciable differences with regard to the colorimetric parameters, both at four and seven months while cutting force showed greater variability, though modest in the *capicolli* at four months.

Tests relative to odour, taste and savouriness (Figs 6, 7 and 8) tended to manifest small differences among the companies for the *capicolli* matured to four months, while for those at seven months an accentuation in these differences was noticeable, and, furthermore, an inversion of judgement regarding taste. The *capicollo* at seven months from company T emerged as being more pleasing compared to the other two, notwithstanding the fact that at four months from the same company had emerged as the least preferred. Savouriness, as was to be expected, was greater in the *capicolli* at seven months, with company T prevailing over the other two for both maturation periods. Lastly, with regard to odour, the judgements of the evaluators favoured companies F and R with respect to company T, both at four and seven months.

CONCLUSIONS

Chemical analysis showed a high protein content (26.31% in the *capicolli* matured to four months and 29.30% for those at seven months) and in contrast, a decidedly low level of fat (8.18% at four months and 9.43% at seven months). From the comparison between the different companies, it emerged that chemical composition for the *capicolli* at four months manifested less variability, above all for humidity, ash, collagen and salt. In those at seven months maturation, on the other hand, apart from their having greater variability, there was in particular an appreciable difference in humidity and protein content between company T and the other two and in lipid content between *capicollo* F and the samples R and T.

These results, with particular reference to the protein and lipid levels, place the product examined in a position of favour compared to other cured cold cuts, above all with regard to the importance which is today attributed to the health quality of food products. In conclusion, therefore, the cured hind leg of pork "*Capicoddho Azze Anca*" emerges on the whole as a cured cold cut of excellent nutritional quality which is highly acceptable to the consumer, both at four and seven months of maturation, but with a greater preference expressed for those which are less matured. Furthermore, we would like to underline also that this cured cold cut, given the age old artisan method used in its production, offers the consumer, apart from its taste and quality, also the flavour of a tradition.

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Figure. 1. Chemical characteristics of *Capicoddho Azze Anca* at four months of maturation.

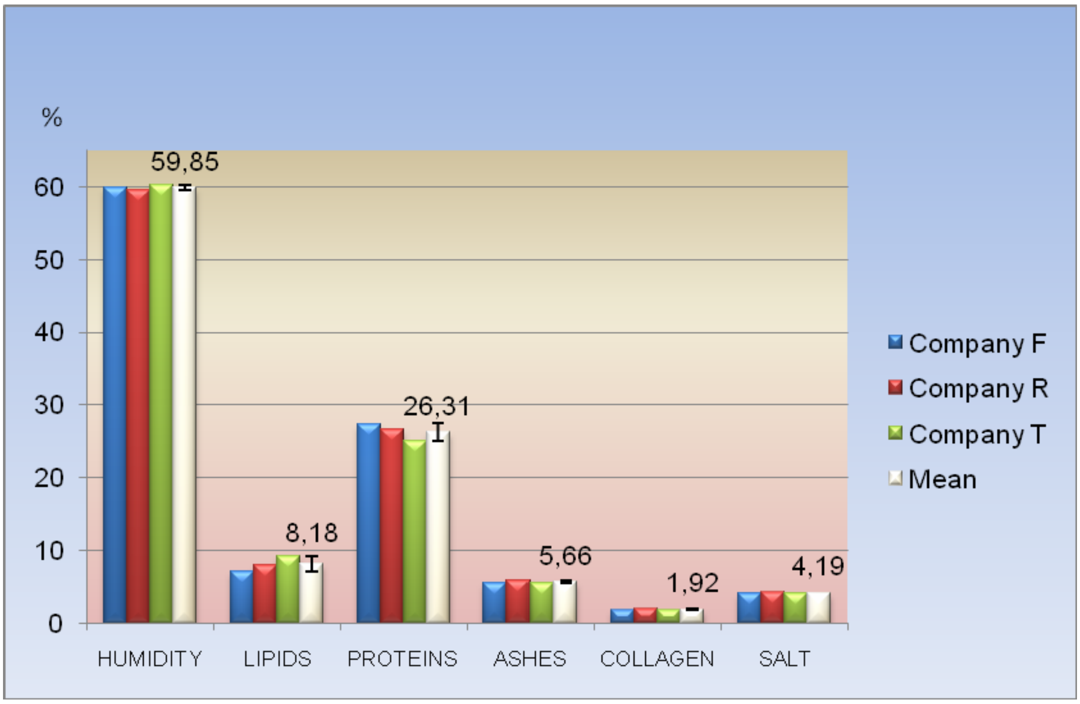


Figure 2. Chemical characteristics of *Capicoddho Azze Anca* at seven months of maturation.

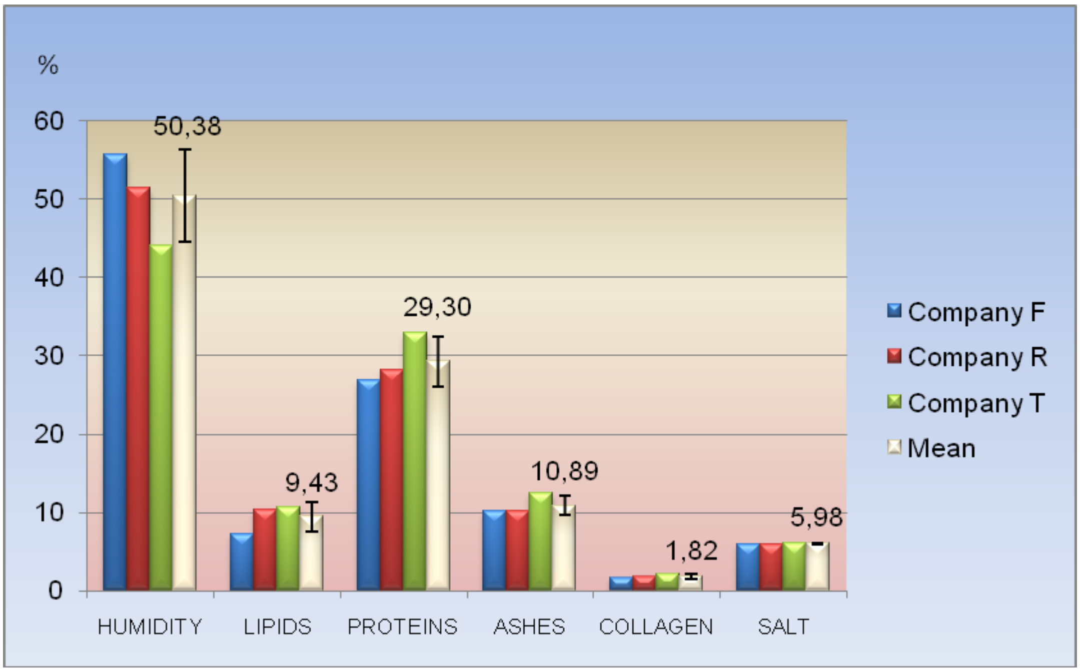


Figure 3. Rheological characteristics of *Capicoddho Azze Anca* at four and seven months of maturation – Test WBS.

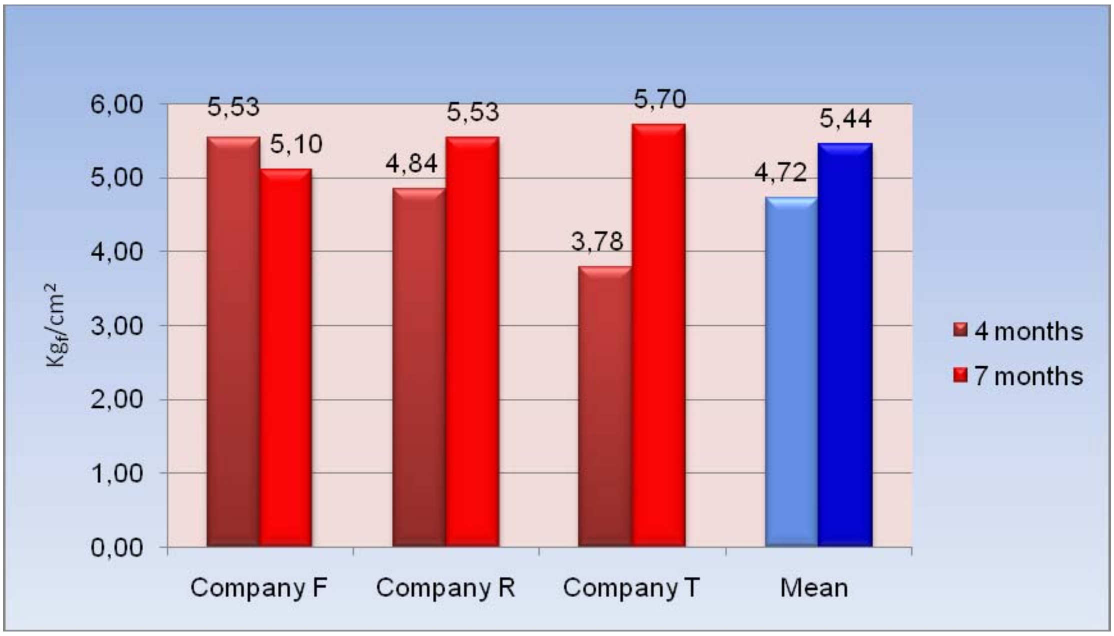


Figure 4. Colorimetric characteristics of *Capicoddho Azze Anca* at four months of maturation

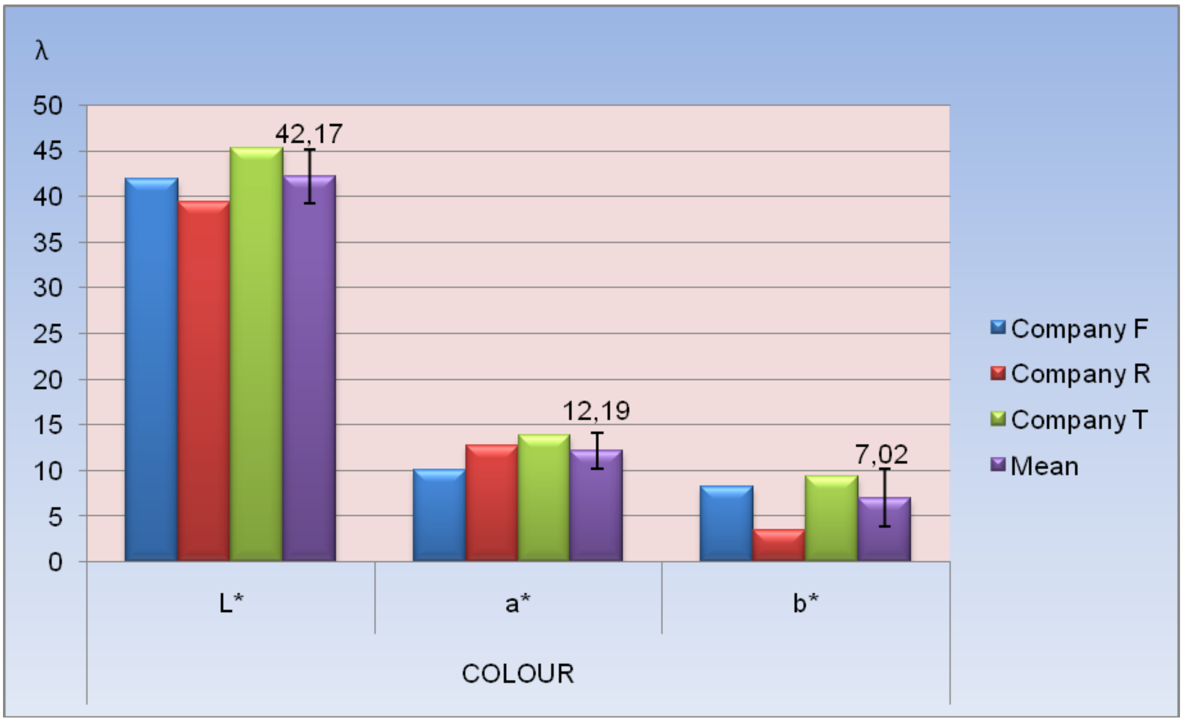


Fig. 5. Colorimetric characteristics of *Capicoddho Azze Anca* at seven months of maturation

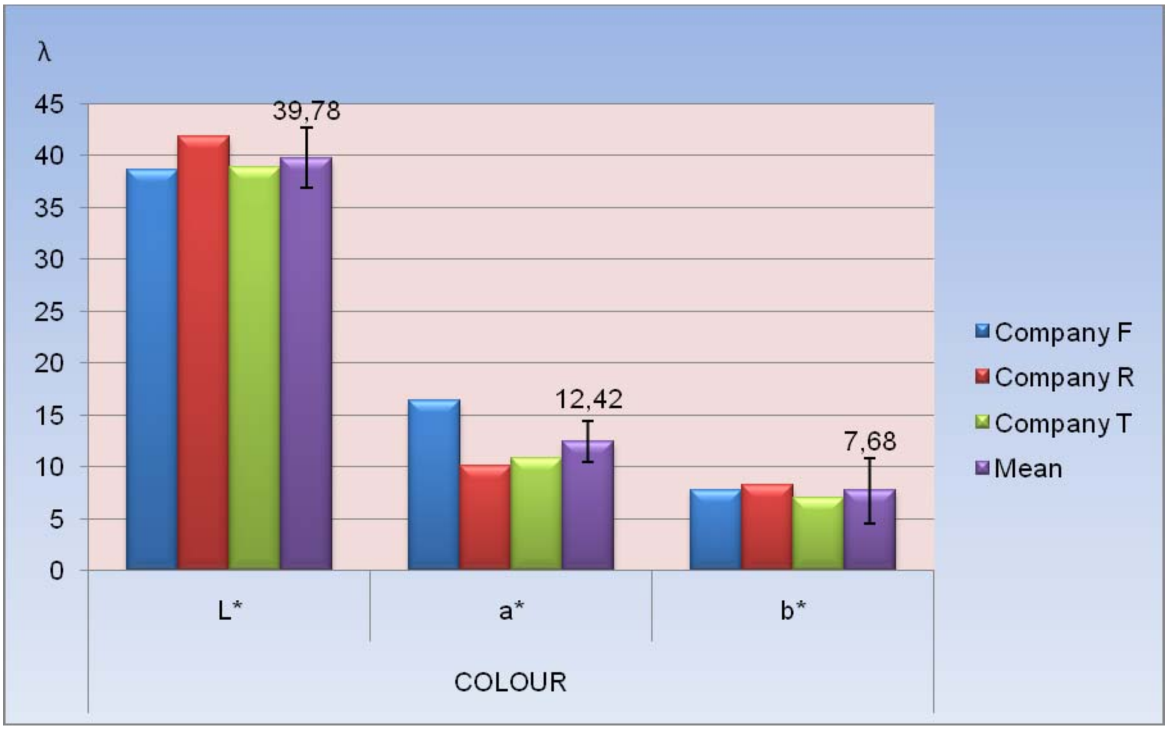


Fig. 6. Odour of *Capicoddho Azze Anca* at four and seven months of maturation

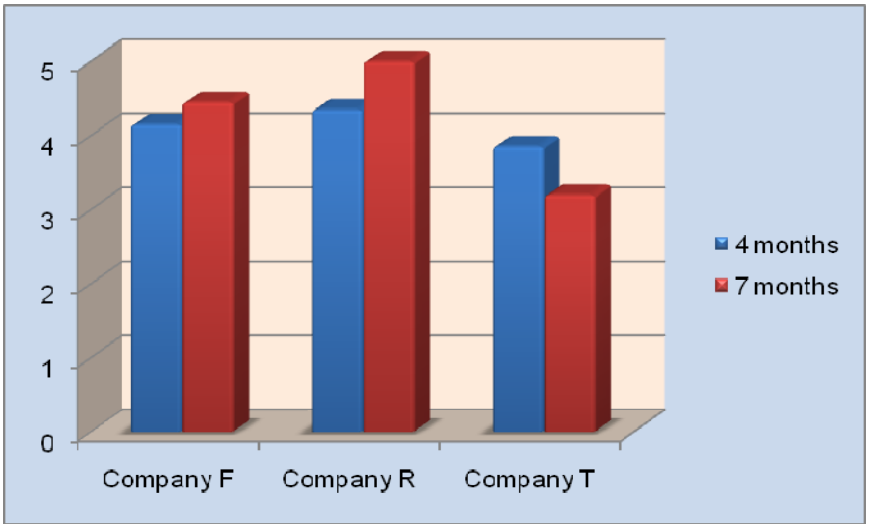


Fig. 7. Taste of *Capicoddho Azze Anca* at four and seven months of maturation

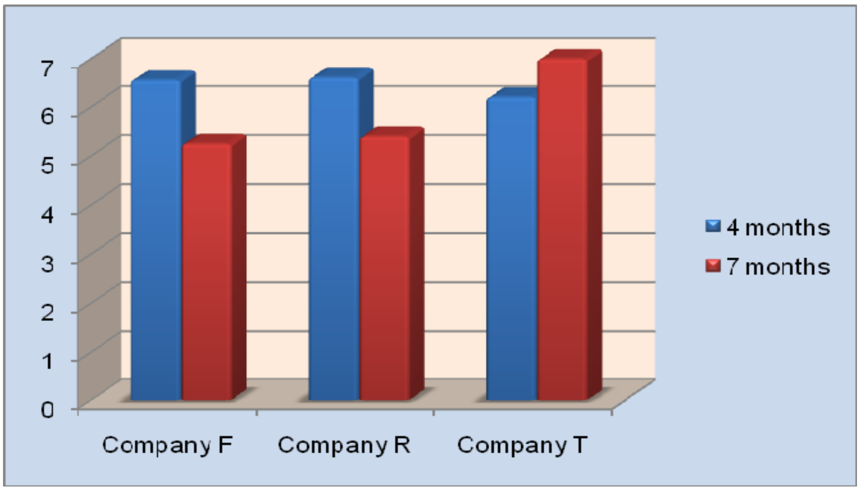
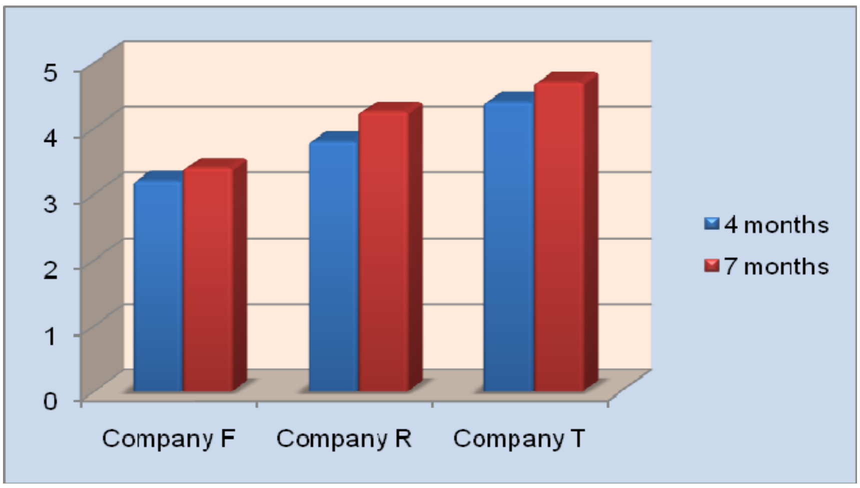


Fig. 8. Savouriness of *Capicoddho Azze Anca* at four and seven months of maturation



LIPIDIC AND AROMATIC FRACTIONS OF "CAPICODDHO AZZE ANCA", A CHARACTERISTIC CURED HIND LEG OF PORK *CAPICOLLO* PRODUCED IN GREEK-CALABRIAN AREA

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SUMMARY – "Capicoddho Azze Anca" – a cured hind leg of pork *capicollo* – is a characteristic cold cut, originating in the ancient Greek area of Calabria (Italy), where it is still produced today in a homemade and traditional manner using the muscles of the thigh and natural flavourings. The lipidic and aromatic fractions of 2 *capicolli*, taken from normal businesses in production, have been examined, at 4 and 7 months of maturation (±15days), for each one of 3 companies. At 4 months of maturation, the average percentage values for saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were respectively 37.66, 43.98 and 18.35, thus revealing a discrete presence of MUFA and PUFA and, consequently, a low saturated/unsaturated ratio (SFA/UFA): 0.61. At 7 months of maturation, the average percentage values for SFA, MUFA and PUFA were respectively 35.05, 41.28 and 23.66, while the SFA/UFA ratio, at 0.54, emerged as being lower than that at 4 months. The ratio between polyunsaturated ω6/ω3, taking into consideration the average values among the three companies, was lower in the *capicolli* at 4 months (14.13 *vs* 18.34). The analysis of the flavoured fraction in general manifested, among other things, a considerable presence of terpenes (limonene and β-pinene) and sesquiterpenes (β-caryophyllene), traceable to the spices used in the preparation phase.

Key Words: Calabrian local product, *capicollo* quality, *capicollo* lipidic fraction, *capicollo* aromatic characterization.

INTRODUCTION

The territory of the Grecanic area is located in the most southern part of the Province of Reggio Calabria (Italy), at the extreme tip of the Italian peninsula, along the valley of the Amendolea river and the Siderone and San Pasquale torrents. It has always been the cradle of the "hellenophonic" Calabrian linguistic minority and custodian of ancient and unchanged popular traditions which have the flavour of the cultural splendours of Magna Graecia (Rohlfs, 1933; Norwich, 1974; Ficarra, 1995; Cudia, 1999). In this area, in which pig breeding is very widespread and has deep roots, a variety of characteristic cured cold cut meats are produced, still prepared today according to "sacred" artisan methods and using recipes handed down over generations with the passion and the pleasure of those who leave to their descendants a piece of their history and of their culture.

The "Capicoddho Azze Anca" is one of these cured cold cuts, produced using the muscles of the hind leg and natural spices, among which, in particular there are wild fennel seed and red chilli pepper, which are characteristic of Calabria. In the context of policies aimed at the rediscovery and valorisation of characteristic products which are being manifested in all the regions of Italy, and considering the fact that this cured cold cut is highly renowned *in loco*, but literally unknown outside its area of production, we feel that it certainly deserves to enjoy greater prominence. In this experimental work, we have sought to study its lipidic and aromatic fractions, with a view to establishing an initial qualitative platform on which subsequent improving interventions can be based and which would be usefully indicative for the productive chain in this sector. In the production of the *capicollo* "Azze Anca" the thigh muscles of "Large White Italiana" X "Nero Calabrese" half breeds are used. From these, three portions are obtained. The first, which is usually of larger dimension, is composed of the *semitendinosus* and *longus vastus* muscles. The second is formed largely from the *semimembranosus*, and the third, which is more round, from the *vastus medialis*, *vastus lateralis* and *rectus femori*.

MATERIAL AND METHODS

For each of three companies, indicated with the letters F, R, and T, representative of the production territory, two hind leg *capicolli* were taken from normal businesses in production. The first, at 1 Kg ± 100g, was composed of *semimembranosus* muscle and was matured to four months (±15 days); the second, at 1,5 Kg ± 100 g, was composed of *semitendinosus* and *longus vastus* muscles and was matured to seven months (± 15 days). Each sample had been conserved in a vacuum pack at a temperature of 2-4° C before the analysis was carried out.

Lipidic fraction

Analytical procedure

Extraction of lipids was carried out using 20g of each sample, homogenized in 10 volumes of a chloroform/methanol mixture 1/1 v/v (200ml of mixture) (Folch *et al*., 1957). The sample was maintained at a temperature of 60°C for 20’. After cooling, 5 volumes (100 ml) of chloroform were added (final mixture ratio for chloroform/methanol: 2/1). Subsequently 5 volumes (100 ml) of a solution of KOH 1N were added, shaken vigorously and left to rest in the refrigerator overnight so as to favour perfect separation of the phases.

The following day, using a separating funnel, the lower phase was removed and subsequently, the solvent was evaporated in a rotating evaporator. The fat thus obtained was esterified using a solution of 2N methanolic potash. The fatty acid methyl esters (FAME) thus obtained were analysed using the gas chromatography method. Gas chromatography analyses were carried out using a Shimadzu GC-2010 instrument with flame ionization detection (FID).

Compounds were separated on 100 m x 0.25 mm I.D. fused-silica columns coated with a 0.20 µm film bonded CP-SIL 88 (VARIAN). The non-polar column was maintained at 150° C for 5’ after injection, then programmed at 3° C per min to 240°C, which was maintained for 25’. Split injection was conducted with a split ratio of 1:100; helium was used as carrier gas. The injector temperature was 250°C and the detector temperature 270°C. Total running time for each sample was 60’. The peaks were identified by their retention times compared to those of known standards (FAME mix Supelco). Some individual components could be identified by co-injection of pure compounds.

Aromatic fraction

Analytical procedure - The extraction of headspace volatile compounds was effected using a SPME device (Supelco, Bellefonte, PA), using a 50/30 mm fibre, divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). Before the analysis, the fibres were preconditioned in the injection port of the GC as indicated by the manufacturer. For each experiment, 2g of *capicollo* were minced and weighed into a 15 ml headspace vial and sealed with a PTFE-faced silicone septum (Supelco, Bellefonte, PA, USA).The vial was left at 50°C in a thermo block for a duration of 20’ to equilibrate its headspace. A SPME fibre was then exposed to the headspace for 30’. The compounds absorbed by the fibre were identified and quantified by means of gas chromatographic analysis using MS detectors (Marco *et al*., 2004).

Identification and quantification of volatile compounds - The compounds absorbed by the fibre were desorbed from the injection port of the gas chromatograph (GC 17A Shimadzu) for 10’ at 250° C in split mode 1/30. The compounds were separated in a SE-52 capillary column (Mega, 30m, 0.25mm I.D., film thickness 0.25µm). The GC was equipped with an QP 5050A mass selective detector (Shimadzu). Helium was used as carrier gas with a linear velocity of 37.3cm/s. The GC oven temperature programme began when the fibre was inserted and held at 40°C for 10’, ramped to 150°C at 3°C per min, then to 250 at 30°C per min and to 250°C for 5’. The total run time was 65‘ and the GC-MS interface was maintained at 270°C. Mass spectra were obtained by electron impact at 70 eV, and data was acquired across the range 35–400uma (Elmore *et al*., 2000). The compounds were identified by comparison with mass spectra from a library database (Nist 21 and 121), Adams library and by comparison with authentic standards. The results are expressed as means of three replicates for each experiment.

RESULTS AND DISCUSSION

The lipid component of all the *capicolli* analysed produced an acidic spectrum both at four and seven months of maturation (Tables 1 and 2), in which saturated fatty acids were identified from C10 to C21, the monounsaturated C14, C17, C18, C20 and the polyunsaturated C18:2n6c, C18:2n6t, C18:3n3, C20:3n3, C20:3n6 and C20:4n6. Among the saturated, those most represented were palmitic (C16:0), with average percentage values 22.21 and 21.42 and stearic (C18:0), with average percentage values of 12.75 and 10.72, at four and seven months of maturation respectively.

Among the monounsaturated, oleic acid stands out (C18:1n9c), with an average presence at 41.18% and a minimum value of 36.88% in the sample from company F and a maximum of 43.71% for company T, found in the *capicolli* at four months of maturation, for which greater variability among the companies was noted in comparison to the *capicolli* at seven months of maturation, which, in their turn, presented an average oleic acid content of 38.60%, with a minimum of 37.63% for company R and a maximum of 39.15% for company T. Polyunsaturated were represented almost exclusively by linoleic acid (C18:2n6c), both at four and seven months (16.10% *vs* 21.69%), with, however, values which tended to be higher and less variable in the latter period of maturation. This situation is reflected by what is reported by Shahidi *et al*. (1986) and Schliemann *et al*. (1987), who state that oleic acid and linoleic acid were the two most abundant unsaturated fatty acids in all samples and, in general, in pork fat. The other most represented polyunsaturated fatty acid was linolenic acid (C18:3n3) with average values both at four and seven months of 1.08% *vs* 1,01%, comparable with the percentages which are normally found, for example, in lard. The ratio between the polyunsaturated ω6/ω3 (Tables 3 and 4), considering the average values among the three companies, was lower in the *capicolli* at four months (14.13 *v*s 18.34), while if we compare the results of the single companies, within each maturation period, there emerges, on the one hand, a variability which is limited at seven months and which is higher among those at four, while on the other, there is an appreciable difference between the value for company T and those of the other two, in *capicolli* at a lesser stage of maturation. The saturated/unsaturated ratio (Tables 3 and 4) emerges as comparable by and large to those of cured hams, with, in particular, values of 0.65 and 0.66 for the samples at four months, respectively for companies F and T.

Table 1. Fatty acids composition of *Capicoddho Azze Anca* at four month of maturation.

Fatty acid (%)		Company			
	Class	F	R	T	Mean
Capric Acid (C10:0)	SFA	0.05	0.05	0.09	0.06
Lauric Acid (C12:0)	SFA	0.06	0.05	0.08	0.06
Myristic Acid (C14:0)	SFA	1.08	0.96	1.26	1.10
Myristoleic Acid (C14:1)	MUFA	0.01	0.01	0.02	0.01
Pentadecanoic Acid (C15:0)	SFA	0.09	0.08	0.06	0.08
Palmitic Acid (C16:0)	SFA	22.08	20.40	24.13	22.21
Palmitoleic Acid (C16:1)	MUFA	1.56	2.49	2.61	2.22
Heptadecanoic Acid (C 17:0)	SFA	0.50	0.42	0.31	0.41
Cis-10-Heptadecenoic Acid (C17:1)	MUFA	0.32	0.41	0.28	0.33
Stearic Acid (C18:0)	SFA	14.42	10.54	13.29	12.75
Oleic Acid (C18:1n9c)	MUFA	36.88	42.95	43.71	41.18
Linolelaidic Acid (C18:2n6t)	PUFA	0.07	0.04	0.02	0.04
Linoleic Acid (C18:2n6c)	PUFA	19.22	18.10	10.99	16.10
Arachidic Acid (C20:0)	SFA	0.23	0.20	0.18	0.20
Linolenic Acid (C18:3n3)	PUFA	1.09	0.87	1.28	1.08
Cis-9-eicosenoico (C20:1)	MUFA	0.36	0.23	0.13	0.24
Heneicosanoic Acid (C21:0)	SFA	1.04	0.90	0.46	0.80
Cis-8, 11, 14-Eicosatrienoic Acid (C20:3n6)	PUFA	0.16	0.19	0.20	0.18
Cis-11,14,17-eicosatrienoic Acid (C20:3n3)	PUFA	0.23	0.17	0.12	0.17
Arachidonic Acid (C20:4n6)	PUFA	0.57	0.96	0.78	0.77

Table 2. Fatty acid compositions of *Capicoddho Azze Anca* at seven month of maturation.

Fatty acid (%)	Class	Company			Mean
		F	R	T	
Capric Acid (C10:0)	SFA	0.05	0.07	0.05	0.06
Lauric Acid (C12:0)	SFA	0.05	0.07	0.06	0.06
Myristic Acid (C14:0)	SFA	1.00	1.20	1.39	1.20
Myristoleic Acid (C14:1)	MUFA	0.01	0.01	0.03	0.02
Pentadecanoic Acid (C15:0)	SFA	0.09	0.08	0.07	0.08
Palmitic Acid (C16:0)	SFA	20.86	21.69	21.72	21.42
Palmitoleic Acid (C16:1)	MUFA	1.73	1.74	3.07	2.18
Heptadecanoic Acid (C 17:0)	SFA	0.30	0.31	0.29	0.30
Cis-10-Heptadecenoic Acid (C17:1)	MUFA	0.26	0.26	0.19	0.24
Stearic Acid (C18:0)	SFA	11.79	11.36	9.02	10.72
Oleic Acid (C18:1n9c)	MUFA	39.02	37.63	39.15	38.60
Linolelaidic Acid (C18:2n6t)	PUFA	0.02	0.02	0.03	0.03
Linoleic Acid (C18:2n6c)	PUFA	21.45	22.24	21.38	21.69
Arachidic Acid (C20:0)	SFA	0.04	0.17	0.14	0.11
Linolenic Acid (C18:3n3)	PUFA	1.02	1.07	0.94	1.01
Cis-9-eicosenoico (C20:1)	MUFA	0.26	0.25	0.26	0.25
Heneicosanoic Acid (C21:0)	SFA	1.12	1.04	1.11	1.09
Cis-8, 11, 14-Eicosatrienoic Acid (C20:3n6)	PUFA	0.15	0.16	0.21	0.17
Cis-11,14,17-eicosatrienoic Acid (C20:3n3)	PUFA	0.23	0.19	0.21	0.21
Arachidonic Acid (C20:4n6)	PUFA	0.55	0.43	0.67	0.55

Table 3. SFA, MUFA, PUFA and their ratios of *Capicoddho Azze Anca* at four month of maturation.

	Company			
Class	F	R	T	Mean
SFA	39.54	33.60	39.86	37.66
PUFA	21.34	20.32	13.40	18.35
MUFA	39.12	46.09	46.74	43.98
w6 PUFA	20.02	19.28	12.00	17.10
w3 PUFA	1.32	1.03	1.40	1.25
w6/w3 ratio	15.17	18.65	8.56	14.13
SFA/UFA ratio	0.65	0.51	0.66	0.61

Table 4. SFA, MUFA, PUFA and their ratios of *Capicoddho Azze Anca* at seven month of maturation.

	Company			
Class	F	R	T	Mean
SFA	35.30	35.99	33.86	35.05
PUFA	23.43	24.11	23.45	23.66
MUFA	41.27	39.90	42.69	41.28
w6 PUFA	22.17	22.85	22.30	22.44
w3 PUFA	1.26	1.26	1.15	1.23
w6/w3 ratio	17.63	18.09	19.31	18.34
SFA/UFA ratio	0.55	0.56	0.51	0.54

The analysis of volatile compounds in the *capicollo* "Azze Anca" through solid-phase micro-extraction has not been previously reported. Given that the selection of an appropriate fibre depends on the compounds and the food to be analysed, and due to previous experience with other foods, we selected a DVB/CAR/PDMS fibre.

Results obtained for the qualitative and quantitative analysis of the oils, both at four and seven months of maturation, are shown in table 5. In total, 35 compounds were classified according to their most likely origin. Typical chromatograms of SPME/GC are shown in Fig 1.

To understand the formation of flavour in *capicolli* it is important to identity the origin of the volatile compound. The flavour characteristics of *capicolli* are thought to result from a combination of microbial activities, auto-oxidation processes, and spices.

In this regard, our *capicolli* manifested a very extensive pattern of volatile components and, for some of them, marked differences were found, both at four and seven months of maturation.

They were mainly aldehydes and terpenes - above all monoterpenes - which together accounted for at least 90% of the total amount of desorbed compounds.

If we consider the group of terpenes, which, as is known, derive from added spices, it is clear, both at four and seven months, that there is a difference of presence among the *capicolli* from the three companies (Table 5). In particular, examination of the most represented terpenes shows, above all for some of these (β-pinene, limonene, fenchone and p-allil anisole) a considerable degree of variability. This, we feel, could be due to the fact that since *Capicoddho Azze Anca* is an exclusively home-made product, and it is not subject to standardisation in terms of quality and quantity as far as regards the mixture of flavourings added during its preparation.

Among the terpenes identified, in particular α-pinene, δ-3 carene, limonene, p-cimene, anethole, p-allil anisole and cineol, derived from the spices used, in particular black pepper and fennel. Limonene is found in many essential oils of spices and is particularly abundant in black pepper which also contains α-pinene, sabinene and β-cariophillene.

Fenchone, anethole and p-allil-anisole are particularly abundant in wild fennel. Differences in terpenic fraction in samples depend on different periods of maturation.

The other compounds were substances of neoformation derived from lipid oxidation during maturation. These compounds are important indicators of the maturation of meat products (Meynier *et al*., 1999).

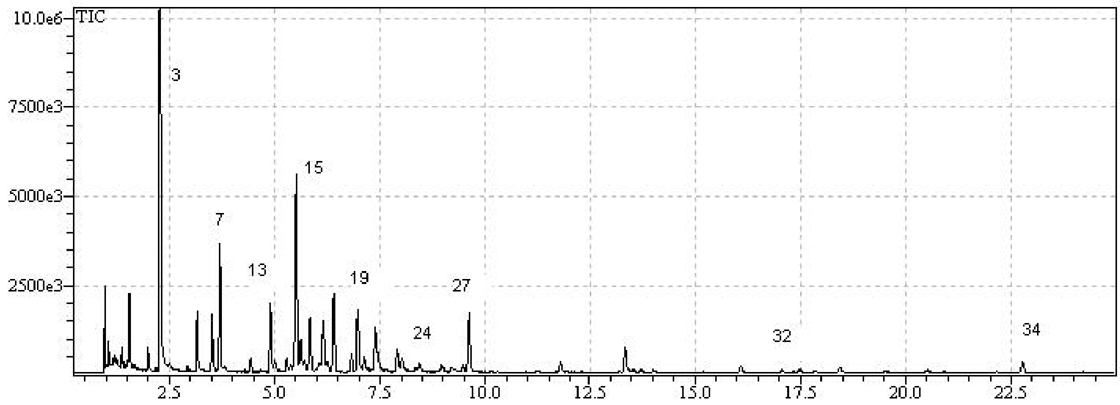
The low amount (12.91% *vs* 19.64% a four and seven months respectively) in samples T can be attributed to an incomplete maturation of the *capicolli*.

The predominant aldehydes detected (Table 5) were hexanal, which imparts a green odour, and then nonanal and heptanal. Nonanal is a product of the auto-oxidation of oleic acid, while hexanal, 2-nonenal and 2,4, decadienal are the major volatile oxidation products of linoleic acid (Shahidi and Pegg, 1994; Paleari *et al*., 2004).

Table 5. Volatile compounds identified in *Capicoddho Azze Anca* at 4 and 7 months of maturation.

	4 months			7 months		
Compound (%)	F	R	T	F	R	T
Spices						
α-Pinene	0.30	0.60	1.02	0.19	0.58	0.87
Sabinene	tr	tr	0.19	tr	0.24	0.35
β-Pinene + 1-Octen -3-Olo	7.67	9.37	0.34	5.69	9.99	1.13
α-Phellandrene	0.82	0.57	tr	tr	0.60	0.19
δ-3-Carene	2.93	1.63	0.30	1.00	3.83	1.04
P-Cymene	0.70	0.64	0.48	0.37	3.77	0.43
Limonene	3.66	2.74	9.33	2.87	3.77	10.57
Cineol	tr	tr	1.50	tr	tr	1.45
Fenchone	1.41	7.45	7.32	1.41	0.49	8.40
Linalol	0.89	0.10	0.51	1.06	0.37	0.51
p-Alli-Anisole	0.25	5.96	65.11	17.13	1.77	54.71
Anethole	0.26	1.02	tr	tr	0.17	tr
β-Caryophyllene	3.37	0.16	1.00	3.68	0.79	0.73
Aldehydes						
Butanal 3-Metil + Pentanal	2.89	1.58	tr	3.66	2.40	0.33
Hexanal	30.54	37.65	0.65	30.92	43.29	6.54
2-Hexenal	0.26	0.17	tr	0.14	0.18	tr
Heptanal	8.64	4.96	0.27	6.79	4.71	1.10
2-Heptenal	2.53	2.52	tr	2.33	3.21	0.47
Benzaldehyde	0.86	0.44	tr	1.04	0.63	0.34
Octanal	9.59	3.74	tr	4.76	2.60	tr
Benzeneacetaldehyde	1.06	1.81	9.70	2.92	2.21	4.85
2-Octenal	1.31	1.51	tr	1.10	1.37	0.39
Nonanal	11.39	4.04	0.61	4.46	3.49	0.89
2-Nonenal	0.63	0.43	tr	0.45	0.64	tr
Decanal	0.31	0.35	0.31	0.48	0.18	0.39
2-Decenal	0.63	0.47	tr	0.49	0.40	tr
2.4-Decadienal	0.23	0.50	tr	0.20	0.34	tr
Stearaldehyde	0.64	0.09	1.07	0.63	0.07	0.21
Alcohols						
1-Pentanol	1.29	1.03	tr	1.21	0.96	0.31
1-Hesanol	0.78	4.55	0.12	1.29	2.28	0.13
Heptanol	1.23	0.39	tr	0.71	0.47	0.12
1-Octanolo	1.05	0.75	tr	0.57	0.60	tr
Ketones						
2-Heptanone	0.66	1.66	0.18	0.65	2.13	3.31
2.3 Ottandione	1.12	1.21	tr	1.54	1.49	0.26
2-Nonanone	0.12	tr	tr	0.22	tr	tr

Figure 1. Typical gas chromatographic traces of aroma volatiles of *Capicoddho Azze Anca* using SPME.



CONCLUSIONS

The analysis of the lipid fraction did not produce remarkable differences, either among the three companies or between the two periods of maturation. It did, however, reveal some characteristics which were of considerable interest. Particularly noteworthy, from the point of view of health and nutrition, was the presence of linoleic acid which is noted for its anti-cancerogenous qualities as well as its properties of stimulation of the immune system and regulation of the metabolism. Analysis of the aromatic fraction indicated a substantially positive progress in maturation and an appropriate addition of spices/flavourings for companies F and R, while revealing conditions which were a little different for company T. As regards this last, although further examination is undoubtedly necessary, the data emerging from this first analysis would suggest a situation which is anomalous in terms of maturation and the flavourings used, which were probably added in large quantities and ground too finely. We are convinced, however, that this is a question which is merely technological and that precise intervention in this company would in all probability eliminate the anomaly encountered, thus returning its product to the sphere of qualitative standards which are characteristic of the other *Capicoddhi Azze Anca*.

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SAMPLE PREPARATION FOR FATTY ACID DETERMINATION OF IBERIAN PIG ADIPOSE TISSUE: CHOPPED *VS* CRUSHED SAMPLES

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SUMMARY –. Previous research has demonstrated the ability of Near Infrared Spectroscopy (NIRS) to accurately predict the fatty acid profile of melted Iberian pig adipose tissue. This study compares two methods of sample preparation before melting, by their ability to extract fatty acids from samples. A total of 20 animals were used (10 from the so called “Cebo” category and 10 from the “Bellota” category). A sample of adipose tissue was taken from each half carcass, in accordance with the guidelines given in the Order PRE/3844/2004. Samples taken from the left half of the carcass were chopped with a knife into 0.5 mm pieces and those from the right side of the carcass were minced using an Ultra Turrax Heidolph Diax 900 homogeniser. A microwave oven was used to extract fat from samples. The main fatty acid profile was determined by NIRS equations. No differences were observed in the fatty acid composition of the melted fat extracted by the two methods in any of the two carcasses categories. Given that chopping the samples is much simpler and quicker, this method is recommended to obtain fat from subcutaneous adipose tissue for the determination of fatty acids.

Key words: Iberian pig, adipose tissue, NIR Spectroscopy, loin, fatty acids

INTRODUCTION

Industry constantly defines new quality control methods as part of their ongoing quality management. These methods should be not only reliable but also fast in order to obtain results quickly. Gas chromatography has been traditionally used in the Iberian pork sector, to control the quality of the carcasses through the determination of fatty acid content in their subcutaneous fat layers. In order to do this, it is necessary to previously obtain a fat sample from the adipose tissue.

The official testing methods for oil and fat in meat and meat products were established when the quality norms for Jamon Iberico (cured Spanish hind leg ham), Paleta Iberica (cured Spanish front leg ham) and Caña de Lomo Iberica (cured Spanish pork loin) were passed in 1981 by Royal Decreed (1083/2001). Moreover NORM PRE/3844/2004 was published in 2004, defining the ways in which samples could be taken from Iberian pigs and the method of analysis for the determination of the composition of fatty acids from the lipids in the subcutaneous adipose tissue of the Iberian Pig.

According to this NORM sample could be obtained either by dissolvent or by melting then in a microwave. Also this NORM regulates the determination of fatty acids through gas chromatography, but it contemplates the possibility of doing it using a precision analysis technique with a different scientific approach.

Since 1992, the Department of Animal Production (ETSIAM) of the University of Cordoba has been working on and testing NIRS to predict fatty acid profiles in the adipose tissue of the Iberian Pork (De Pedro et al., 1992; Garcia-Olmo et al., 2000). Garcia-Olmo in particular indicates that there is an error in the prediction equations for the main fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid) in melted fat, similar to the one in existing in the gas chromatography method.

Given the complex methodology when crushing the subcutaneous fat samples of the Iberian Pig in order to extract the fat, this study intended to show that there is a possibility of replacing this method with another, in which the samples simply need to be chopped with a knife and later heated in a microwave oven, without affecting the fatty acid content, which determines the quality of the carcass.

MATERIAL AND METHODS

Twenty Iberian pigs were used for this study, ten of which were fed whit commercial feedstuff in their final feeding phase and 10 which only consumed acorns and forage (montanera). A subcutaneous fat sample was taken from the entire carcass by making an incision next to the spine about 10 cm from the tail, as required by Order PRE/3844/2004.

The samples were placed in plastic bags, labelled and put into a portable cooler, after which they were transported at -20ºC until the moment of the analysis.

Two sheets with a width of 1cm were taken from the central piece of each sample and the skin and meat were separated. One of the samples was cut into little pieces with a knife, while the other one was crushed in an Ultraturbax Heidolph Diax 900 blender that allows for the preparation of a homogenous mix.

In order to melt the fat, a microwave oven was used according to the methodology proposed by De Pedro et al. (1997), because no reagents are need, it is more environmentally-friendly, faster and does not affect the fat content, something that cannot be avoided when extracting the fat with the help of dissolvent. Once the liquid fat was obtained, 4ml of each sample were taken for analysis.

Fatty acids determination was done using NIRS equations shown in Table 1. Spectral data were recorded from 400 to 2500 nm using a Foss-NIRSystem 6500 scanning monochromator equipped with a spinning module. Samples were analysed by folded transmission using a gold reflectance ring cup with a very narrow pathlenght (0.1 mm, ref. IH-03459).[3] Spectra were collected with The ISI NIRS 3 software ver. 3.11 (Infrasoft International, Port Matilda, PA, USA) was used for calibration development and evaluation according to Mark and Workman (1991) and Shenk and Westerhaus (1995, 1996).

Table 1. Performance statistics for fatty acids prediction

Fatty Acid	N	Mean	Mín/Máx	SD	SEC	R^2	ETVC	r^2
C16.0 (%)	99	20,99	15,81/26,18	1,73	0,44	0,934	0,45	0,933
C18:0(%)	99	10,32	6,08/14,54	1,41	0,31	0,953	0,33	0,946
C18:1(%)	95	52,87	42,75/62,98	3,37	0,23	0,995	0,24	0,995
C18:2(%)	93	9,51	5,93/13,08	1,19	0,16	0,981	0,17	0,979

N: number of individuals that make up the population. SD: Standard deviation. Min/Max: makes reference to the range of the equation. SEC: standard error of calibration. SECV: standard error of cross validation. R^2 and r^2: determination coefficient for calibration and cross validation.

Data were statistically analyse whit SAS software (SAS, 2001) following the model:

$$Y_{ijk} = \mu + A_i + P_j + AP_{ij} + \varepsilon_{ijk}$$

while:
Y_{ijk} = value of the characteristics of sample k of nutrition type i and preparation type j.
μ = general mean value
Ai = effect of feeding system; i=1, 2
Pj = effect of the sample preparation method; j=1, 2.
APij = interaction between the effect of the nutrition and the way it was cut j.
εijk = random error margin.

RESULTS

The composition of the main fatty acids of the subcutaneous fat of the Iberian pig, ordered according to the preparation method of the samples, chopped or crushed, in pigs fed with acorns or with animal feed are presented in Table 2.

Table 2. Mean composition of main fatty acids in Iberian pigs according to diet (acorns or animal feed), and in order of form of sample preparation (chopped or crushed)

FATTY ACID	ACORN				ANIMAL FEED			
	CHOPPED (n=10)		CRUSHED (n=10)		CHOPPED (n=10)		CRUSHED (n=10)	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
C16:0	20,54	0,41	20,65	0,39	23,37	0,32	23,45	0,40
C18:0	9,50	0,38	9,46	0,47	11,45	0,41	11,51	0,45
C18:1	54,73	0,71	54,47	0,77	49,34	0,75	49,11	0,91
C18:2	8,71	0,33	8,84	0,42	8,64	0,25	8,67	0,29

The variance analysis showed the lack of interaction between the way the samples were cut and the type of diet. It also showed that there are no significant differences between the two ways of preparing the samples. Differences were found between the fatty acid contents of pigs fed on a different diet. There were significant differences in linoleic acid content which may have been due to a low content of linoleic acid of the feedstuff.

The results confirmed that samples used to extract fat for the analysis of fatty acid content can be either chopped or crushed in a blender. It is thus a question of preference for each laboratory that carries out the analysis.

However, preparing the samples with a blender is a more complex process due to several reasons: First of all, jars are required to crush the samples which are used for the extraction of the liquid fat. These receptacles need to be cleaned after each batch in order to avoid contamination, which can lead to mistakes. In contrast, chopping the samples can be accomplished with a simple knife.

Chopping the samples therefore seems to be the more adequate method for the preparation of samples, as it makes the process simpler, which is an important factor in the slaughtering process and the carcass quality control.

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EFFECT OF PASTURE ON WOOD ON AROMATIC PROFILE OF SEASONED FAT OF CINTA SENESE PIG

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SUMMARY - The influence of the volatile compounds on the characterization of Cinta Senese seasoned fat was studied using solid phase microextraction (SPME) and Gas Chomatography-Mass Spectrometry. The analysis were carried out on 27 pigs fed three different feeding systems: pasture on acorn (AC), pasture on chestnut (CH) and commercial mixture (CM). About 150 volatile compounds were identified and assigned to 14 chemical families. Compounds were identified by comparing their mass spectra with those contained in the NIST and Wiley libraries and by comparison of Kovats indexes with those reported in the literature. Alifatic hydrocarbons, aldehydes, alcohols, acids and terpenes dominated the volatile compounds. Small amounts of sulphur compounds, ester, ether, ketones, aromatic hydrocarbons and other miscellaneous compounds were also present. The effect of feeding system on the proportion of identified volatiles was tested by an analysis of variance (ANOVA), using GLM procedure. Significant differences of some volatile compounds related to fat oxidation were found among seasoned fat from pigs reared in different feeding systems.

Key words: Cinta Senese pig, seasoned fat, volatile profile, feeding system.

INTRODUCTION

Dry-curing of Cinta Senese hams is a traditional process that leads to a food product with unique flavour. Raw hams are undergone to four stages of seasoning in the course of a long process over 18 months (salting with dry salt, washing, post-salting for salt equalization and ripening-drying). The aroma is due to chemical and biological changes, giving rise to a large number of volatile compounds that contribute to their characteristic flavour. Moreover, compounds coming from feeds and spices also contribute to the final flavour of dry-cured meat products. In fact quality of meat products from Cinta Senese pigs strongly depends on rearing (outdoors or indoors) and feeding system (acorn, chestnut, mixture). Then the aim of this study was to evaluate the effect of pasture on oak plantation and on chestnut grove on aromatic traits of dry-cured ham.

MATERIAL AND METHODS

This study was carried out with thirty Cinta Senese pigs: 10 pigs were reared in paddock and fed commercial mixture (CM), 10 pigs were reared in wood and fed chestnut and grass (CH), 10 pigs were reared in wood and fed acorn and grass (AC). Pigs were slaughtered at 148 kg of live weight, on average. For more details on experimental design see Pugliese *et al.* (2006).,At slaughter, raw hams were cured according to dry seasoning technique of "Prosciutto Toscano". On 14 seasoned hams (5 CM, 5 CH, 4 AC), samples of subcutaneous fat were taken. Volatile compounds of seasoned fat were extracted by headspace-SPME (Ruiz *et al.*, 2001). One gram was weighed into a 4 ml vial and screw-capped with a Teflon-silicone disk. A SPME (Supelco, Bellefonte, PA, USA) fiber coated with carboxen/poly(dimethylsiloxane) (75µm thickness) was inserted into the sample vial and exposed to the headspace. Extraction was performed at 37°C for 30 min in a water bath. Before the analysis the SPME fiber was preconditioned at 280°C for 50 min. in the gas chromatograph injection port. Volatile compounds analysis was performed using a HP-6890-GC series II gas chromatograph (Hewlett-Packard) coupled to a mass selective detector (HP-5973 Network, Hewlett-Packard). Volatiles were separated using a 5% phenyl-methylsilicone (HP-5) bonded phase fused-silica capillary column (50 m

0.32 mm i.d., film thickness 1.05 lm, Hewlett-Packard). Carrier gas was Helium at a flow rate of 1.45 ml min/1. Compounds were identified by comparing their mass spectra with those contained in the NIST and Wiley libraries and by comparison of Kovats indexes with those reported in the literature.

The effect of feeding system on the proportion of identified volatiles was tested by an analysis of variance (ANOVA), using GLM procedure (SAS, 2003).

RESULTS AND DISCUSSION

In the trial about 103 volatile compounds were identified and assigned to the respective chemical families. The main compounds are shown in Table 1. Most of them are derived from the autoxidation of unsaturated fatty acids, although some of them could also derive from amino acids, having a Strecker degradation origin (Ruiz *et al*., 1999). A high number of acids has been detected. This result agrees also with research on Iberian dry-cured ham (Andrés *et al*.,2002). Aldehydes compounds represent the most important chemical family in Cinta Senese seasoned fat; among them hexanal, the main aldehydic compound, derives from the oxidation of n-6 fatty acids, heptanal arises from oleic or linoleic autooxidation and, finally, octanal and nonanal come from oleic acid oxidation. Other compounds from lipid oxidation were also aliphatic hydrocarbons (as pentane and hexane), furans (as furan-2-ethyl) and ketones (as 2-pentanone, 2-propanone, 2,3-pentanedione). About aromatic hydrocarbons, methylbenzene (toluene) could derive from cyclation of unsaturated carboxylic chains produced by lipid degradation (Min *et al*., 1977). The major ester found was ethyl acetate, which is formed through esterification reactions between ethanol and carboxilic acids. It leads to fruity notes and it has a low odour threshold (Muriel *et al.*, 2004).

Other chemical families, such as sulfur compounds (i.e. carbon disulfide), nitrogen compounds (i.e. 2,6-dimethylpirazine and pyridine), derive from Maillard reaction (Ruiz *et al*., 2001); pirazine has characteristic walnut, toasted or potato odours (Meynier *et al*., 1999). The main terpen found is linalool that leads to flowery notes. The accumulation of terpens in fat depots has two possible origins: from animal feeding or from spices added during processing (Muriel *et al*., 2004).

As regard the rearing system effect the significance was found only for ethers compounds, highest in AC group. For the other compounds, the high variability didn’t allow to reach the significance threshold, nevertheless the differences are high in absolute values.

Apart of the significant level it is possible to underline that the highest percentages of aldehydes (2-propenal, 2-propanal, pentanal, hexanal, heptanal) and furans compounds were found in seasoned fat from pigs reared on acorn. These results agree with those reported by Muriel *et al*. (2004) who found higher levels of these aldehydes in dry-cured Iberian hams from pigs pastured on oak wood. Regarding ketons, as 2-propanone, 2,3-pentanedione and aliphatic hydrocarbons, pigs fed chestnut showed the highest percentage.

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Table 1. Effect of feeding system on percentage of volatile compounds in subcutaneous fat of dry-cured ham of Cinta Senese pig.

COMPOUNDS*	KI	Id**	FEEDING SYSTEM			P	SEM
			CH	AC	CM		
ACIDS			21.01	14.95	23.67	0.27	8.00
acetic acid	595	MS	1.68	2.95	3.02	0.74	3.00
butanoic acid	788	MS	1.04	0.59	0.97	0.62	0.71
butanoic acid, 3-methyl	841	MS	0.41	0.32	0.33	0.90	0.33
hexanoic acid	974	MS	4.00	4.11	3.39	0.95	3.60
hexanoic acid ethyl ester	995	MS	1.27	2.17	1.63	0.21	0.70
tetradecanoic acid	1755	MS	1.01	0.32	0.67	0.44	1.50
hexadecanoic acid	1977	MS	3.80	1.62	4.32	0.23	2.30
ALCOHOLS			6.46	9.16	8.24	0.41	3.01
ethanol	<500	MS	5.65	8.49	6.79	0.46	3.28
ALDEHYDES			27.31	40.87	29.57	0.14	9.81
acetaldeyde	<500	MS	2.83	1.29	1.61	0.30	1.52
2-propenal	510	MS/KI	2.47	3.65	1.86	0.48	2.16
pentanal	698	MS/KI	1.05	3.35	3.07	0.28	2.29
hexanal	800	MS/KI	17.01	23.20	17.39	0.37	6.89
heptanal	900	MS/KI	1.07	2.77	1.16	0.35	1.85
octanal	1000	MS/KI	0.64	0.69	0.45	0.50	0.32
ALIFATIC HYDROCARBONS			21.04	11.20	19.61	0.47	12.22
pentane	500	MS/KI	2.94	5.57	2.27	0.10	2.18
hexane	600	MS/KI	2.34	0.10	1.35	0.42	2.43
heptadecane	1700	MS/KI	1.51	0.18	1.42	0.47	1.70
octadecane	1800	MS/KI	1.56	0	2.14	0.46	2.53
AROMATIC HYDROCARBONS			0.30	0.49	0.29	0.56	0.29
benzene, methyl (toluene)	772	MS/KI	0.15	0.32	0.13	0.30	0.19
CHLORIDE COMPOUNDS			0.99	0.88	0.81	0.61	0.28
chloroform	609	MS/KI	0.70	0.51	0.63	0.66	0.31
ESTERS			1.12	1.00	1.13	0.97	0.88
ethylacetate	605	MS/KI	0.90	0.82	0.89	0.98	0.75
ETHERS			0.91	3.34	1.36	0.06	1.41
ethane 1,1 oxybis	520	MS/KI	0.91	3.34	1.36	0.06	1.41
FURANS			0.65	1.14	0.60	0.52	0.74
furan 2-ethyl	704	MS/KI	0.12	0.15	0.23	0.64	0.18
furan 2-pentyl	991	MS	0.43	0.70	0.21	0.36	0.49
KETONES			13.74	11.68	9.33	0.72	8.57
2-propanone	512	MS/KI	5.19	6.07	3.53	0.61	3.84
2-pentanone	684	MS/KI	4.41	2.38	2.48	0.66	3.80
2,3- pentanedione	696	MS/KI	2.67	1.34	0.99	0.49	2.26
2-heptanone	890	MS/KI	0.50	0.70	0.55	0.59	0.29
NITROGENS COMPOUNDS			0.66	0.56	0.73	0.76	0.34
pyridine	748	MS/KI	0.06	0.04	0.09	0.72	0.10
pyrazine 2,6 dimethyl	912	MS/KI	0.34	0.33	0.31	0.97	0.20
SULPHUR COMPOUNDS			2.14	1.68	1.53	0.86	1.80
carbon disulfide	544	MS/KI	0.85	0.97	1.19	0.94	1.63
TERPENS			0.06	0.19	0.08	0.36	0.13
linalool	1097	MS/KI	0.06	0.19	0.08	0.36	0.13
ANHYDRIDS			4.04	3.14	3.26	0.52	1.28
carbon dioxide			4.04	3.14	3.26	0.52	1.28

* Expressed as area percentage of total volatile compounds identified.
** MS = Mass spectrum tentatively identified using NIST and Wiley library. KI = Kovats index in agreement with literature.

EFFECT OF SIRE BREED IN CINTA SENESE CROSSBREEDS: CHEMICAL, PHYSICAL AND SENSORIAL TRAITS OF FRESH AND SEASONED LOIN.

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SUMMARY - Seventeen Cinta Senese (CS), 18 Large White x Cinta Senese (LWxCS) and 16 Duroc x Cinta Sense (DxCS) pigs were employed. Animals of each genetic type were distributed in two groups according to rearing system: outdoors (OUT) and indoors (IN). Pigs were fed the same commercial diet. Pigs were slaughtered at 153 kg of l.w., on average. Chemical-physical and sensory analysis were performed on fresh and on seasoned *Longissimus lumborum*. On fresh meat LWxCS showed the highest moisture and ash content while DxCS showed the lowest protein content and higher fat percentage than LWxCS. LWxCS exhibited the lowest value of *a** while CS had the highest value of *L*.* Drip loss was higher in LWxCS than in CS. DxCS showed higher shear force on raw meat than LWxCS and lower shear force on cooked meat than CS. On seasoned loin CS scored the highest redness while LWxCS had the lowest marbling, oiliness and aroma. CS showed higher value of *a** than LWxCS and the highest value of *b*.* As regard rearing system, on raw meat IN determined higher values of *a** and lower of cooking loss; on seasoned loin OUT scored higher for *L** and *b** and lower for marbling.

Key words: crossbreed, Cinta Senese pig, meat quality, seasoned loin.

INTRODUCTION

Local pigs, as Cinta Senese breed, are generally linked to extensive farming where they can offer products characterised by high specificity. Nevertheless, for a better knowledge of their potential, it is advisable to evaluate their performances also under intensive management (Legault *et al.*, 1996; Serra *et al.*, 1998) and in comparison to selected breeds (Labroue *et al.*, 2000; Legault *et al.*, 1996; Serra *et al.*, 1998).

In Southern Europe local pigs are often crossed with selected breeds to exploit additive and non-additive genetic variance, as in the production of Iberian x Duroc (Carrapiso *et al.*, 2003) and Corsican x Large White (Coutron-Gambotti *et al.*, 1998). Crosses between Cinta Senese sows and Large White boars have been historically produced by Cinta Senese farmers.

The aim of this work was to study physical, chemical and sensory traits of fresh and seasoned loin in the Cinta Senese breed compared to their crosses with Large White and Duroc breeds.

MATERIAL AND METHODS

Seventeen Cinta Senese (CS), 18 Large White x Cinta Senese (LWxCS) and 16 Duroc x Cinta Senese (DxCS) pigs were employed. Animals of each genetic type were allotted in two homogenous groups according to rearing system: outdoors (OUT) in paddock of 5 ha, and indoors (IN) in box.

Pigs were fed the same commercial diet and were slaughtered at 153 kg of live weight, on average. Chemical-physical and sensory analyses were performed on both fresh and seasoned (2 months) *Longissimus lumborum*. Chemical and physical traits were analysed by analysis of variance, using the GLM procedure (SAS, 2003) following this model : $Y_{ijklm} = \mu + B_i + R_j + S_k + D_l + (BxR)_{ij} + b(X_{ijklm}) + E_{ijklm}$ where B, R, S, D are breed, rearing system, sex, day of slaughter and X is live weight. Sensory traits were analysed by MIXED procedure: $Y_{ijklmn} = B_i + R_j + S_k + T_l + V_m + (BxR)_{ij} + E_{ijklmn}$ where T and V are taster and day of tasting.

RESULTS AND DISCUSSION

In chemical traits of fresh meat (table 1) LWxCS showed the highest moisture and ash content while DxCS showed the lowest protein value and higher fat percentage than LWxCS. This confirms the well-known high marbling of muscle of Duroc breed (Lo *et al.*, 1992; Blanchard *et al.*, 1999; Lebret *et al.*, 2001).

As regard physical traits of fresh meat (table 2), drip loss was higher in LWxCS than in CS. The result can be explained by a lower content of intramuscular fat in this group. DxCS showed higher shear force (SF) on raw meat than LWxCS and lower SF on cooked meat than CS. CS had the lowest value of L* while LWxCS showed the lowest value of a* probably due to the higher myoglobin content of local breeds (Ruiz *et al.,* 2004).

On seasoned loin, CS (table 3 and 4) confirmed the higher value of a* than LWxCS and showed the highest value of b*. Also the subjective evaluation (panel test) scored the highest redness of CS. LWxCS had the lowest marbling, oiliness and aroma, probably because of the lower content of fat. As regard rearing system IN determined higher values of a* and lower of cooking loss on raw meat; on seasoned loin, OUT pigs showed higher values of L* and b* and scored lower of marbling and higher for flavour persistence.

Table 1 – Chemical composition (%) of fresh *Longissimus lumborum*

	Breed			Rearing System		RSD
	CS	DCxCS	LWxCS	IN	OUT	
Moisture	70.16 a	69.88 a	71.30 b	69.99	70.90	1.63
Protein	22.88 a	22.31 b	22.83 a	22.85 a	22.49 b	0.60
Ether Extract	5.73 ab	6.67 a	4.68 b	6.06	5.32	1.90
Ash	1.15 ab	1.01 a	1.30 b	1.17	1.13	0.24

a,b, $P<0.05$

Table 2 – Physical traits of fresh *Longissimus lumborum*

	Breed			Rearing System		RSD
	CS	DCxCS	LWxCS	IN	OUT	
Drip loss (%)	1.93 a	2.66 ab	3.42 b	2.60	2.74	1.47
Cooking loss (%)	20.13	19.57	21.07	18.49 a	22.03 b	4.51
Free water (cm^2)	9.41	10.44	8.95	9.63	9.57	2.11
Shear force raw meat	10.09 ab	11.02 a	9.60 b	10.09	10.39	1.47
Shear force cooked meat	11.55 a	9.64 b	11.32 ab	10.58	11.09	2.39
Ph45	6.51	6.56	6.43	6.46	6.55	0.26
pH24	5.69	5.62	5.61	5.60 a	5.68 b	0.13
L*	45.38 a	49.58 b	49.14 b	47.85	48.22	2.68
a*	12.24 a	11.80 a	10.43 b	12.00 a	10.97 b	1.62
b*	2.99	3.60	3.34	3.40	3.22	1.03

a,b, $P<0.05$

Table 3 – Characteristics of seasoned *Longissimus lumborum* (p<0.05)

	Breed			Rearing System		RSD
	CS	DCxCS	LWxCS	IN	OUT	
L*	38.68	39.59	38.28	37.87 a	39.84 b	1.69
a*	12.29 a	11.72 ab	10.92 b	11.81	11.48	1.40
b*	5.76 a	4.54 b	4.42 b	4.37 a	5.43 b	1.15
MDA mg\kg	0.33	0.31	0.25	0.30	0.30	0.12

a,b, P<0.05

Table 4 – Sensory characteristics (0 to 100 scale) of seasoned *Longissimus lumborum*

	Breed			Rearing System		RSD
	CS	DCxCS	LWxCS	IN	OUT	
Redness	44.73 a	34.53 b	37.01 b	39.55	37.97	13.64
Brownness	28.16	29.02	29.37	28.66	29.05	13.24
Colour uniformity	44.86	40.37	45.22	41.42	45.55	16.24
Marbling	34.55 a	38.00 a	21.96 b	35.00 a	28.01 b	13.71
Oiliness	24.62 a	24.13 a	18.32 b	21.28	23.43	12.70
Aroma intensity	39.43 a	40.38 a	34.46 b	38.37	37.81	13.77
Saltiness	45.04	43.34	40.48	42.36	43.54	13.00
Mastication resistance	28.29	30.39	31.67	29.66	30.57	13.94
Juiciness	37.33	38.06	36.56	37.28	37.34	14.18
Flavour persistence	43.21	41.95	42.70	41.27 a	43.96 b	13.82
Aftertaste	7.52	7.30	6.66	7.19	7.13	10.16
Global pleasure	42.52	43.94	43.54	43.97	42.70	14.91

a,b, P<0.05

CONCLUSIONS

The cross between Cinta Senese and some selected breeds could be a good way to improve and increase the value of the local breed. Especially it seems that DxCS crosses could reach a quality level of the meat not inferior to the one of Cinta Senese purebreed, showing similar values of several analysed parameters. DxCS and CS scored a more intense colour and a lower SF than LWxCS. Rather than LWxCS, DxCS pigs could bear products of high quality and have better growth performances than the local breed. The quality of both raw and seasoned meat was not affected by the tested rearing systems.

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DETERMINATION OF HYDROXYPROLINE IN PORK SAUSAGES AND DRY CURED BEEF PRODUCTS BY NIRS TECHNOLOGY EMPLOYING A FIBRE–OPTIC PROBE

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SUMMARY - In the present work a study was made of the use of NIRS (Near Infra-red Spectroscopy) technology with a remote reflectance fibre optic probe for the analysis of hydroxyproline in cured pork sausages (*salchichón and chorizo*) and dry cured beef (*cecina*). The presence of the amino acid hydroxyproline was used as an analytical criterion to assess the amount of collagen from conjunctive tissues *versus* lean meat (muscle). The equation developed for a total of 80 samples (*salchichón*, *chorizo* and *cecina*) allowed the determination of the hydroxyproline content within a range of 0% - 0.74%. The multiple correlation coefficient (RSQ) and standard error of prediction corrected (SEP(C)) obtained were respectively 0.772 and 0.05%. The predicted values of hydroxyproline in unknown samples using NIRS technology and applying the fibre optic probe directly on the sample without previous treatment were comparable to those obtained using the chemical method.

Key words: Hydroxyproline, determination, pork sausages , NIRS.

INTRODUCTION.

Collagen is a fibrous protein that forms conjunctive tissue. It is mainly made of glycine and very large amounts of proline and hydroxyproline and is one of the few proteins containing hydroxylysine (Bailey & Litght, 1989). The amount and the tissue composition of connective tissue in beef affect its texture (Sadowska, 1992) and the sensory characteristics of the meat (Seidman, 1986).

Hydroxyproline, a non-essential amino acid constituent of proteins, is mainly found in connective and bone tissue and forms up to 10% of collagen molecules (Stanley, 1983).

Cured pork sausages are products made by grinding meat and fat, with or without the lights, to which authorized spices, additives and condiments are added, after which the sausages are subjected to a process of curing, with optional smoking. The term *cecina* (dry cured beef) is used to refer to non-ground meat and butchery scraps subjected to the appropriate action of common salt and other authorized products used in the salting process, either in solid form or in brine, thereby guaranteeing the conservation of the meat for later consumption. The presence of hydroxyproline is used as an analytical criterion to assess the amount of collagen from the skin, tendons, cartilages and ligaments, thus enabling the ratio of lean meat (muscle) and conjunctive tissues (collagen, which is cheaper) used in the elaboration of the mixtures for *chorizo*, *salchichón* and *cecina* to be determined.

In Spain, cured sausage meats are classified in different categories (*extra*, 1st, 2nd and 3rd), depending on their composition in moisture, protein, fat, hydroxyproline, and soluble and insoluble carbohydrates. The maximum amount of hydroxyproline in cured pork sausages (order of 7th February, 1980 (B.O.E., 21/3/1980), which regulates the quality directives concerning these products) should be between 0.6% and 0.9% m/m, depending on the category of the product.

The determination of hydroxyproline levels in meat and meat products(for example, sausages,) is essentially based on colorimetric evaluation at 560 nm of the derivative formed by hydroxyproline and p-dimethylamino-benzaldehyde, which involves prior hydrolysis of the proteins in acid medium and oxidation of the hydroxyproline with chloramine. In comparison with this slow and tedious method (with a mean duration of 9-10 hours), the literature contains references to the use of near infra-red spectroscopy for the determination of hydroxyproline, expressed as collagen in beef (Isaksson & Hildrum, 1990; Mitsumoto et al., 1991; Calvo et al., 1997; Alomar et al., 2003, and Prieto et al., 2006), although with poor prediction results. NIRS technology has been used in cured pork sausages for the determination of the major parameters (moisture, fat and protein) and for the discrimination between Iberian swine and non-Iberian pork sausages (Ortiz-Somovilla et al., 2005; 2007).

NIRS technology with a remote reflectance fibre optic probe has been used in the determination of parameters of interest in products derived from Iberian pork such as protein and fat infiltrated into pork loin, fatty acids in pork loin and subcutaneous tissue (González-Martín et al., 2002; 2003; 2005)

The aim of the present study was thus to obtain NIRS calibrations for the instantaneous and simultaneous prediction of hydroxyproline in cured pork sausages and dry cured beef (*cecina*) by means of the NIRS technique, using a remote reflectance fibre optic probe applied directly on the homogenised sample, thus allowing expedient quality control of these products.

MATERIAL AND METHODS.

Samples and chemical analyses.

In the present study, 92 samples of cured pork sausages made in Spain (80 samples used in the calibration set and 12 in the external validation set) were studied. The calibration set was made up of 50 samples of Iberian *chorizo extra* and non-Iberian *chorizo extra*; 20 samples were of Iberian *salchichón extra* and non-Iberian *salchichón extra* and 10 samples were of *cecina* from León (NW Spain). Before chemical determination of hydroxyproline, the samples were homogenised in a Knifetec 1095 blender, Foss Tecator. Official meat and meat product analysis methods were used (MAPA, 1993), in accordance with the AOAC standard (AOAC, 1996). All determinations were carried out in duplicate and the results are expressed in g/100g. The chemical compositions in hydroxyproline of the different types of sausage from the calibration set are shown in table 1, together with the mean and standard deviation.

Table 1. Content of hydroxyproline (g/100g) in cured pork sausage (*chorizo* and *salchichón*) and dry cured beef (*cecina*). Calibration sample set.

Type of sausage	Nº of samples	Minimum	maximum	Mean	SD
Chorizo	50	0.23	0.74	0.33	0.12
Salchichón	20	0.19	0.55	0.34	0.11
Cecina	10	0.13	0.33	0.20	0.09

NIR Spectroscopy

A Foss NIRSystem 5000 with a standard 1.5 m 210/7210 bundle fibre-optic probe, Ref nº R6539-A, was used. The probe employs a remote reflectance system and uses a ceramic plate as reference. The window is of quartz with a 5 x 5 cm surface area, measuring reflectance in the IR zone close to 1100-2000 nm. Spectra were recorded at intervals of 2 nm, performing 32 scans for both the reference and samples. The NIR spectra were recorded by direct application of the fibre-optic probe onto the ground samples. To minimise sampling error, all samples were analysed in triplicate. The software used was Win ISI 1.50, installed on a Hewlett-Packard Pentium III computer.

Statistical analyses

Hydroxyproline quantification was accomplished by means of the modified partial least squares (MPLS) regression method to obtain the NIR equations for all the parameters studied. Partial least squares (PLS) regression is similar to principal component regression (PCR), but uses both reference data (chemical, physical, etc.) and spectral information to form the factors useful for fitting purposes (Martens & Naes (2001)). MPLS is often more stable and accurate than the standard PLS algorithm. In MPLS, the NIR residuals at each wavelength, obtained after each factor has been calculated, are standardised (dividing by the standard deviations of the residuals at each wavelength) before calculating the next factor. When developing MPLS equations, cross-validation is recommended in order to select the optimal number of factors and to avoid overfitting (Shenk & Westerhaus (1996).

RESULTS AND DISCUSSION

Spectral information.

The NIR spectra were recorded by applying the fibre optic probe directly onto the samples of homogenised sausage, recording all the samples. Figure 1 shows the set of NIR spectra used in this work. The NIR spectra reflect the variability of the samples and their composition, which allows the hydroxyproline component to be quantified. Figure 2 shows the spectrum of a sample obtained directly with the fibre-optic probe and one of the mathematical treatments that afforded optimum values for the calibrations of hydroxyproline (using Detrend for scattering correction and the 1st derivative 1,4,4,1; this nomenclature is explained above).

Quantitative analysis. Calibration equations

Initially, the spectra were treated using principal component analysis (PCA). The spectral variability explained was 99.62%, and 8 principal components were required. The risk of mistakes in the equations under practical conditions is very low or almost nil when using the standardised H-statistic (Mahalanobis distance) during routine analysis of unknown samples. This indicates how different the spectrum of the unknown sample is from the average spectrum in the calibration set. Samples with an H-value greater than 3 may be considered as not belonging to the population from which the equations were developed, and in this case the equations should not be used to make any prediction. In the present case, 6 samples were removed.

Figure 1. Spectra of 92 samples (cured pork sausage (*chorizo* and *salchichón*) and dry cured beef (*cecina*).

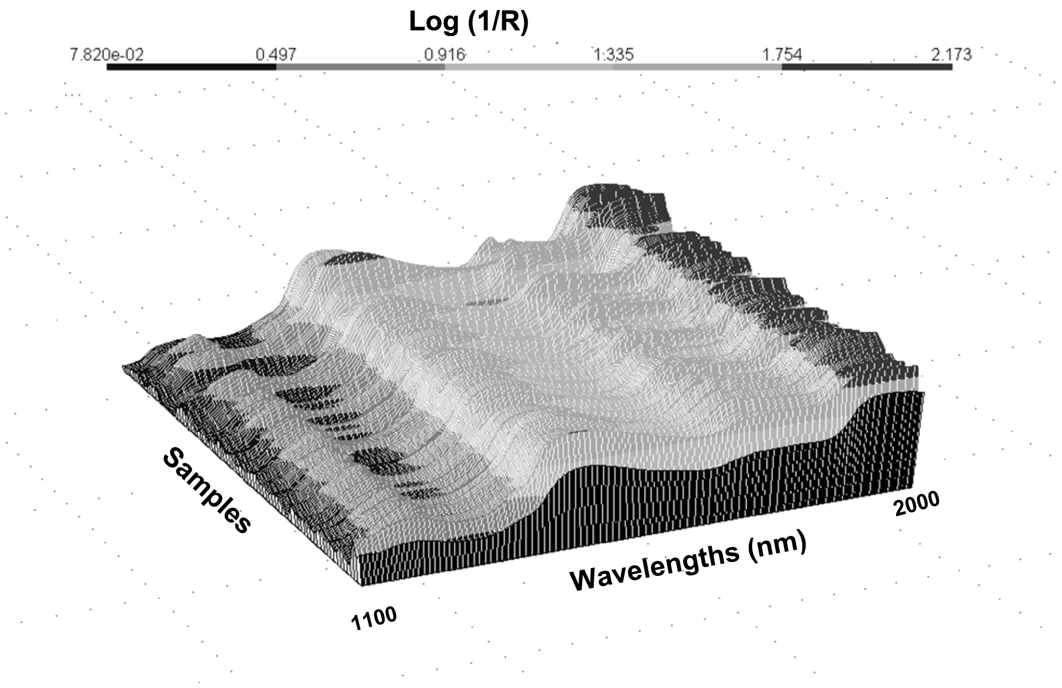
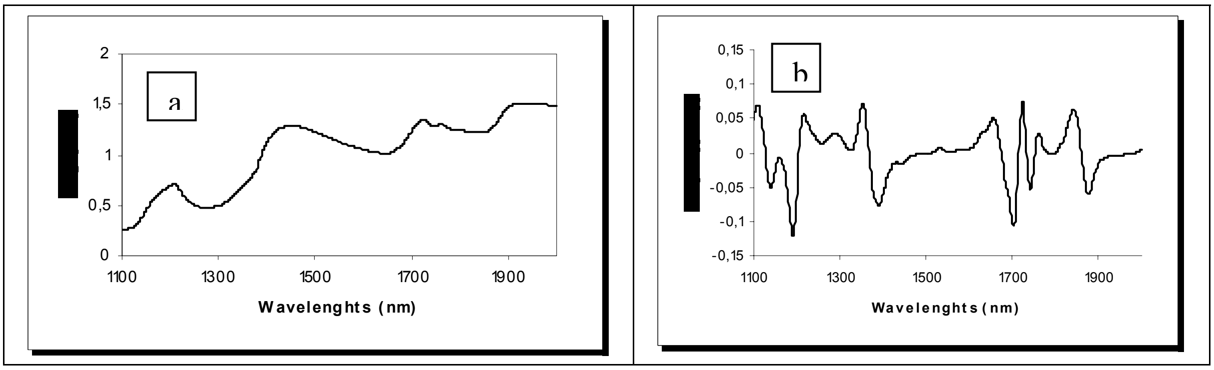


Figure 2. NIR Spectrum (a) and corrected spectrum using 1st derivative and Detrend (b).



Calibrations were performed by modified partial least squares regression (MPLS). Using the T≥2.5 criterion, samples that were different from the population owing to chemical criteria were removed from the set; according to this chemical criterion, 4 samples were removed from the calibration set.

The statistical parameters of the calibration equations for hydroxyproline in *chorizo*, *salchichón* and *cecina* samples are shown in Table 2, where N is the number of samples used to obtain the calibration equation after removing the samples for spectral (H criterion) or for chemical reasons (T criterion). The best of the different mathematical treatments, concentration ranges, and standard deviations for hydroxyproline are also shown. The results obtained indicate that it is possible to determine hydroxyproline in cured pork sausages (*salchichón*, *chorizo*) and dry cured beef products (*cecina*) from Spain using NIRS with a fibre optic probe applied directly to the previously homogenised sample.

Table 2. Calibration statistical descriptors for the NIR determination of hydroxyproline

N	Mean	SD	Est. Min	Est. Max	RSQ	SEC	SECV	RPD
70	0.35	0.11	0	0.65	0.772	0.05	0.07	2.2

N : Number of samples; SD: standard deviation; RSQ: multiple correlation coefficients; SEC: standard error of calibration; SECV: standard error of cross-validation; RPD: ratio performance deviation : SD(ref) / SEP(NIR).

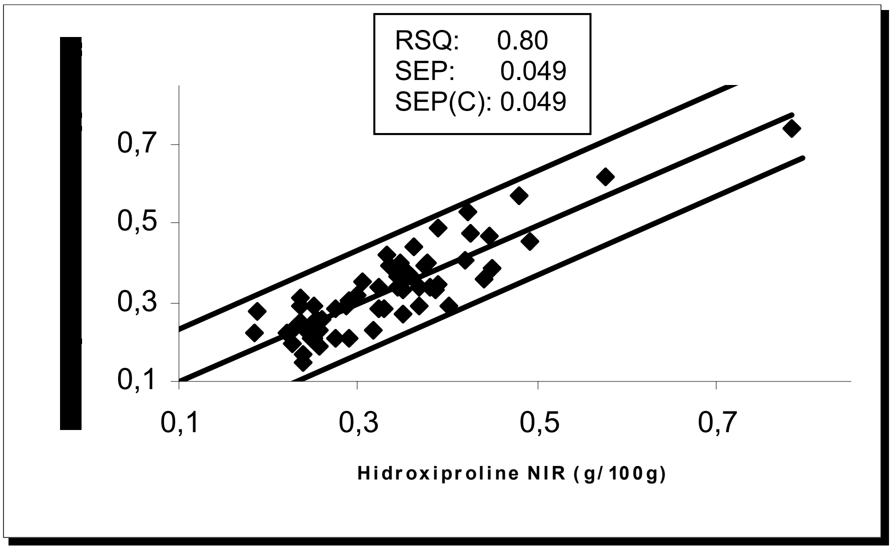
Validation

Internal validation (prediction)

Model evaluation was performed by cross-validation. In this method, the set of calibration samples is divided into a series of subsets. In all cases, cross-validation was performed by splitting the population into six groups. Of these, 5 were taken for the calibration set and one for the prediction set. The process is repeated as many times as there are sets, such that all pass through the calibration set and the prediction set. With this process, the model was validated and its prediction capacity was checked. The graphics in figure 3 show the correlations of the values obtained at the laboratory with respect to those predicted by NIR with a fibre optic probe for hydroxyproline in cured pork sausage and dry cured beef.

The prediction capacity of the model was assessed using the RPD (ratio performance deviation) parameter, which is defined as the relationship between the standard deviation of the chemical method (SD ref) and that of prediction in the NIR model (SEP) (Williams and Sobering, 1996). If the RPD value is ideally greater than 2.5, it is assumed that the calibration model is adequate but lower RPD values can be attributed either to a narrow range of the reference values (giving a small SD) or to a large error in the estimation (SECV) compared to SD. The RPD value obtained with the samples used for hydroxyproline determination (2.2) show that the NIR equation obtained can be applied to unknown samples. This RPD value is lower than 2.5; in this case, this can be attributed to a narrow range of the reference values (giving a small SD). From these data it may be inferred that the NIRS technique, using a fibre-optic probe, offers an alternative for the determination of hydroxyproline in *chorizo*, *salchichón* and *cecina*.

Figure 3. Comparison of the reference values with the values predicted by the calibration equations.RSQ: multiple correlation coefficients; SEP: standard prediction error; SEP(C): standard prediction error corrected by the bias.



External validation

The robustness of the analytical method was confirmed by applying it to 12 samples for the external validation. The procedure was as follows: spectra were recorded in triplicate and the spectral mean was taken. Then, the calibration equations obtained during the work were applied and the predicted values were compared with those obtained later on using the reference methods. The NIRS and chemical methodologies were compared for the four additives using Student's t-test for paired values with these samples. The level of significance obtained was 0.47 at a minimum level of significance of 0.05. It may thus be deduced that the predicted values of hydroxyproline using NIRS technology are comparable to those obtained using chemical methods.

CONCLUSIONS

The equations developed using NIRS technology together with the use of a remote reflectance fibre-optic probe allow the content of hydroxyproline to be determined in a range between 0% and 0.65%. The results of this study should allow the control and the detection of fraudulent practices in the elaboration de cured pork sausages and dry cured beef by direct application of the fibre-optic probe on the sample.

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SESSION 5
QUALITY ASSURANCE AND TRACEABILITY FOR TYPICAL MEAT PRODUCTS

NEAR INFRARED SPECTROSCOPY SENSORS FOR QUALITY ASSURANCE AND TRACEABILITY OF TYPICAL PORK MEAT PRODUCTS: THE IBERIAN PIG, A STUDY CASE

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SUMMARY –.This paper tries to contribute to the scientific dissemination of the potential of Near Infrared Spectroscopy technology (NIRS) for the quality assessment and traceability of meat products. The chapter does not focus on an exhaustive literature review of the topic, but rather presents a study case, taken from the research experience of the Department, on the use of NIRS for quality and traceability of Iberian Pig products.

Keywords:; Traceability, Quality assurance schemes , Near Infrared Spectroscopy, Typical pork meat products, Iberian pig.

INTRODUCTION

Traceability has been defined by ISO as the ability "to trace the history, application or location of that which is under consideration" (ISO, 2000). Many European countries have developed traceability systems for food in general, and especially, for livestock and their products (Smith *et al.*, 2005). Despite the abundant rise of information about labelling, traceability systems and quality assurance schemes, the effect on consumer trust in meat as a safe and wholesome product is only limited (Gellynck,*et al.*, 2006). In many cases, the label provides a short, non-useful and non understable description and consumers demand more and better identification and transparency on meat quality attributes and traceability. Consumers also demand “that the product is what the label stays that is”.

The increase in the complexity of the food chain and the recent food crisis (i.e.mad cow disease - BSE in 1996 in the UK, dioxin contamination in Belgium in 1999, etc), have made that consumers wish to know about the different stages existing in the food chain, that is production, transformation and distribution (FSA, 2002). Many research studies have demonstrated that consumers are willing to pay for traceability but would pay more if traceability is linked to quality attributes (i.e nutritional and health properties, welfare and environmental production practices, food safety, typical production methods, etc.).

However, production of typical meat products (i.e. out-door production, natural resources feeding, animal breeds preservation, landscape preservation, traditional receipts, healthy properties, etc) are very costly and difficult (if not impossible) to demonstrate. This is well known to both to industrial and consumers. That is why both, industrial and consumers, lack incentive and motivation to implement and to pay for methods to ensure compliance of the products with high quality and safety standards.

At present, there are a huge amounts of analytical methods and technologies (PCR fingerprints, RFID, imaging...) for ensuring traceability and quality assurance systems. However, in practice, most of the industrials use methods based in record-keeping systems (also required for HACCP systems). However, the record keeping step of the HACCP system usually is based in documents and protocols providing information about how the safety of the process is under control, and how corrections are going to be made if a deviation from defined critical limits are observed.

The scientific community should transfer information, to industrial, consumers and theirs associations and to official inspection bodies, on the existence of available scientific knowledge about other low cost methods and technologies that can be useful not only for fulfilling compulsory regulations about product quality, but also, for process self-control, and for increasing the market for a product of traceable and demonstrable differentiated attributes.

Several reviews and chapter books shows the tremendous research efforts aimed to demonstrate the ability of NIRS for the analysis of many quantitative and qualitative attributes of meat and meat derived products (i.e. Prevolnik *et al.*, 2004). Furthermore, Near Infrared Spectroscopy has demonstrated to be an affordable technology for use in traceability, quality and labelling programs in the agro-food sector (Garrido and de Pedro, 2007).

NIRS FOR QUALITY AND TRACEABILITY OF IBERIAN PIG PRODUCTS

Agro-forestry system is a term used to describe land management systems that combine the production of trees, with crops and/or animals preserving local practices to produce high value-added food and non food products. The Dehesa is considered a multiple resource production system based on the harvesting of a variety of products such as meat, milk, eggs, cereals, honey, olive fruits, cork, charcoal, timber, defense against fire, water, landscape, diversity, integration of domestic and wild fauna, shelter for fauna, etc. A well managed Dehesa is one of the best examples of integration between grassland productivity and sustainability (Gastó *et al.*, 1993, EGF, 2006). The “Dehesa” is one example of a complex mediterranean agro-forestry system also called sylvo-pastoral, and it is the natural habitat of the Iberian Pig , IP (Garrido and de Pedro, 2007)

Iberian ham is a luxury cured product obtained from pigs lefts free to graze on acorns in Spanish *dehesa.* Acorn production on the *dehesa* depends on agro-climatic conditions and for this reason Iberian pigs are fed exclusively on acorns and grass at the end of the rearing period, or acorns and grass and variable amounts of concentrates or compound feedingstuffs. The *dehesa* feeding and rearing system is known to impart a special flavour to the IP cured products and in particular top the ham, which commands a premium price with respect to products that are inferior in the eyes of consumers.

Evidence suggests that pigs fed exclusively on acorns during the final rearing period produce the highest-quality final cured products and in particular the highest-priced hams. Certain scientific evidence also confirms that animals fed mainly on acorns and grass have higher contents of oleic acid and lower contents of stearic acid than other animals fed on acorns and compound feeding stuffs, or only on compound feedingstuffs (De Pedro, *et al.*, 1995)

Every year, the Spanish Ministry of Agriculture and Fisheries publishes quality specifications (*Contrato Tipo Homologado*) for Iberian pig products in the National Official Journal (BOE, 2000); these specifications are based, among other variables, on the percentage of palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) in subcutaneous fat, as determined by gas chromatography (GC). The quality specifications allow these pigs to be classified into three commercial categories *("bellota"*, *"recebo"* and *"pienso")*, with different FA contents and prices.

Like other high-quality products (i.e. virgin olive oil, wine), most Iberian ham is now produced under a given PDO (Protected Designation of Origin). PDO covers the term used to describe a foodstuff which are produced, processed and prepared in a given geographical area using recognised know how. The PDOs were created to encourage diverse agricultural production systems, to protect names for misuse and imitation and to help consumers by given the information concerning the specific characteristics of the product (EC, 1992). At present, Iberian ham PDOs, private industries and the Spanish pig producers association have established quality control programmes to determine FA composition in pigs reared each year. However, the high cost and the time required to analyse the samples by GC mean that in practice it is only possible to control the fat representing the mean content of a limited number of animals in a given batch.

The optimum quality of the IP hams (“acorn” category) is reached by keeping a given production system that is mainly based in the breed purity of the animals (at least 75% of IP blood) and consumption of Dehesa natural resources. In other words, during the final growing period (100 to 160 kg; each individual pig must have gained 5 @ (1 @ = 11.5 kg) or at least 60% of the weight at the start of the finishing period (86 to 105 kg) by consumption of acorns (fruits of the *Quercus ilex* spp), grass and other natural resources (Che Man *et al.*, 1998). However, variable agro-climatic conditions cause enormous year-to-year variability in the available natural resources and thus in the number of animals belonging to each of the three commercial categories: "acorn", "recebo" and "concentrates" (animals only feed with compound feed).

Iberian pig hams have a high national and international reputation not only because of its taste and flavor, but also because of health properties (i.e., high oleic acid content) and because of the "organic" nature of the Iberian pig production system. At present, the inspection controls established for the Iberian pig are based upon "on farm" inspector visits and fatty acid analysis done in one fat sample taken from a group of animals from the same producer. That system is very time consuming and expensive, and not objective enough to fulfill the increasing consumer demands from regional, national, and international markets. Another downside of the system is that it does not provide information about the production and quality of each individual ham.

The implementation of traceability, labeling, and/or certification systems is rather more complicated in agro-forestry systems than in industrial production processes. In fact, the understanding and definition of the scientific and technological basis for such control systems overpass individual capabilities of companies and suppliers that offer traceability systems. However, for the Dehesa, and in particular for the IP production system, it is crucial to develop such systems to avoid frauds and to ensure consumer expectations after paying for such a luxurious and expensive product because not only does it taste delicious but also because of its contribution to the consumer's heath and to the environment.

The authors´team at the University of Cordoba (Spain) have spent several years evaluating the potential of NIRS technology for the quality control of Iberian pig products, including its most prestigious product – ham. NIRS has been used both, for ***quantitative*** analysis, that is, prediction of the fatty acids content and for ***qualitative*** analysis or classification of pig carcasses into the three mentioned commercial categories (De Pedro *et al*., 1992, 2007a,b,c; García Olmo *et al.*, 2000, 2004; Luna Delgado *et al*., 2004). Table 1 shows that Near Infrared Spectroscopy accurately grading Iberian pig carcasses according the feeding regime, using calibration equations for the prediction of the major fatty acids of the adipose tissue. The calibration equations have coefficients of determination (r^2) reaching values close to 1 and standard error of cross validation (SECV) values similar to the standard error in the reference method (SEL).

Table 1. NIRS equations to predict weight percentage of fatty acids on Iberian pig fat (Garcia Olmo, *et al*, 2001)

Constituent	Mean	SD	SEC	R^2	SECV	r^2	SEL(1)
C16:0	21.00	1.39	0.23	0.97	0.26	0.97	0.26
C18:0	10.68	1.34	0.24	0.97	0.28	0.96	0.22
C18:1	52.24	2.37	0.24	0.99	0.26	0.99	0.25
C18:2	9.40	1.33	0.13	0.99	0.15	0.99	0.15

(1) Calculated from 20 samples analysed by duplicated by the reference method (GC)

The equations showed in Table 1 were obtained using melted fat. In order to use the full potential of NIRS, calibration equation have been obtained also using intact adipose tissue. Fig 1 shows how NIRS may be used at the slaughter house for carcasses grading, avoiding the need for falt melting prior to NIR analysis.

The author´s team has also developed (see de Pedro *et al*., 2007, in this proceeding books) NIRS models models for carcass grading, based on the near infrared spectral (NIRS) data "per se". NIRS discriminant models obtained with hundreds of samples may correctly classified 100% of the animals fed on commercial feed and only a very low percentage (around 1.5 %) of the animals belonging to the *Bellota* free range feeding system may be misclassified.

Although NIRS technology provides rapid analysis, results are not as readily-available as might be desired due to delays prompted by sample collection, transfer and handling. Works is being done on the development and optimization of NIRS technology for fat measurement both in live pigs – the technique is totally harmless for the animal – and in carcases in the slaughterhouse; this would enable consolidation of a traceability system based on rapid, non-destructive sensors. Choice of instrument and measuring accessories is of key importance for optimizing the collection of high-quality spectra, both in live pigs during fattening and in whole carcases after scalding (De Pedro, *et al.*, 2007b)

CONCLUSIONS

At present, new methodologies, technologies, and research trends are emerging providing a multidisciplinary approach for building more innovative, creative, sustainable, credible, and affordable traceability and labelling program for the Iberian Pig production system (Garrido and de Pedro, .2007). A trans-disciplinary group of researchers and professionals is being launched by the authors´team with the main goal of design and evaluate a methodology based in the use of different spectral complementary sensors, for the management of the Dehesa, using among others, the near infrared region of the electromagnetic spectrum The sensors may be placed in different locations and supports (laboratories, hand-held, aeroplanes, helicopters, satellites). Cooperation with other groups interested in a systemic approach for the study traditional Mediterranean agro-forestry systems and for innovation in technologies aimed to help to cover consumer expectations about quality and safety of traditional Mediterranean agro-forest products is much desired.

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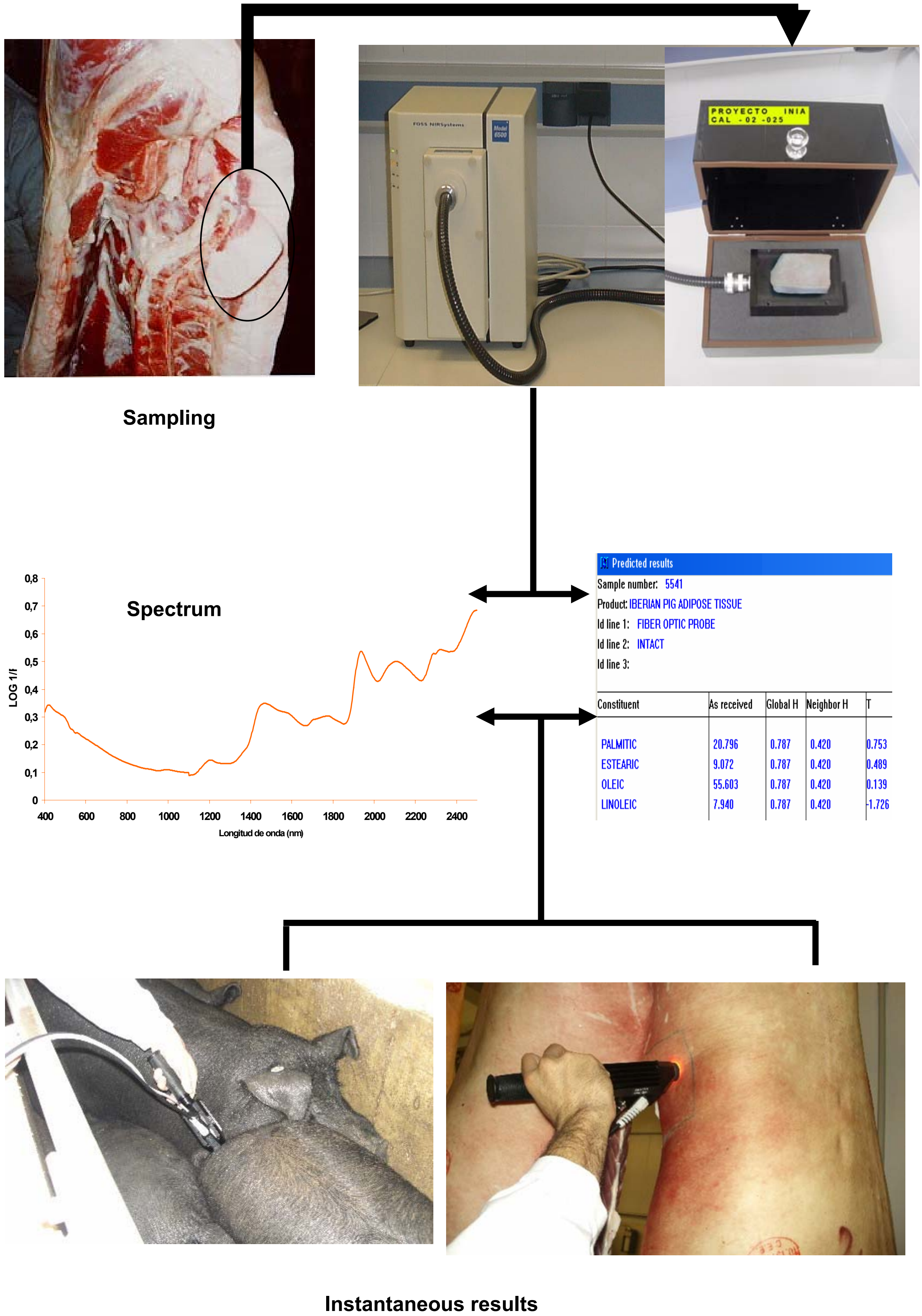
Figure 1. Collection of NIRS spectra in live animals and carcases (de Pedro *et al.*, 2007).



VALIDATION OF FERMENTED SAUSAGES SAFETY WITH THE USE OF A CHECK LIST IN TRADITIONAL WORKSHOPS FROM SOUTH OF PORTUGAL

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SUMMARY - The aim of the work was to evaluate hygiene procedures and manufacture of "chouriço", a fermented dry sausage, by the use of a check list and its relationship with final product safety. Five different traditional workshops in a south region of Portugal were evaluated with a questionnaire concerning Good Hygiene Practices (GHP) and check of critical control point (CCP) and GHP control points (GHP-CP) with a supplementary sampling for direct contact surfaces and final product. All the workshops had a score > 30 in the questionnaire 1st part being considered Sufficient for prerequirements needed to a successful implementation of HACCP. Nevertheless, the less scored workshop on 1st part (34) did not achieve 30 points in the 2nd part due to hygiene indicator high counts and *Listeria monocytogenes* in environmental samples (table and knife), and in final product. This check list seems to be a good tool for safety verification and validation in traditional workshops.

Key Words: Safety, fermented sausages, traditional products, validation

INTRODUCTION

Consumers prefer traditional fermented meat products produced in traditional small units due to their taste and aroma characteristics. The manufacture of traditional sausages is done according to an ancestral know-how related to particular country regions. The major goals of a Food Operator is to produce and put on the market, taste, convenient and safe products in order to establish and maintain a trust relationship with consumer and be rewarded by his effort. Safety and genuineness concept implies that food will not cause harm to the consumer since it is prepared, kept and eaten according to its intended use, nature and origin may be retrieved along the food chain. The safety of traditional fermented meat products produced in small and farmhouse units must be assured and has to be achieved by combination of different hurdles and with different preventive proactive measures assisted by mandatory reactive inspection.

WHO (1999) recognized that small businesses usually have greater difficulties in implementing HACCP and that the Codex *Hazard Analysis and Critical Control Point System and Guidelines for its Application* is developed from the perspective of large food industries and it is not well-adapted to small businesses. It was concluded that governments and professional trade bodies have a clear role to play in facilitating the implementation of HACCP in small businesses and other food businesses with less developed food safety management systems (referred as less developed businesses), and that there is a need to develop specific guidelines for them.

An important consideration in implementing HACCP in small businesses is the recognition that there exists a critical interdependency between HACCP and prerequisite programmes (PRP). Prior to implement HACCP, businesses must be engaged in good hygienic practices (GHP). By first implementing GHP, the difficulties associated with HACCP are minimized and businesses are encouraged to follow a graduated or stepwise approach to HACCP implementation.

Standard requirements of hygiene in the production, handling, packaging, storage and transportation of fermented meat sausages are mandatory to assure a healthful and wholesome supply of such products and the successful design and implementation of a HACCP program.

Layout and operative conditions of premises and equipment coupled with Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP) programs are critical to small meat processing plants, being indicated as pre-requirements to a HACCP plan of fermented meat sausages.

General GHP and GMP codes for different process food categories have been already performed by regulatory food standard organizations (FAO/WHO, 2005). Enterprises must define and elaborate their own GHP and GMP Codes. From the GHP, the hygiene program should be verified and validated to assure its effectiveness.

The aim of this study was to evaluate hygiene procedures and manufacture of “chouriço”, a fermented dry sausage from South Portugal, by a check list and its relationship with safety and stability of final product.

MATERIAL AND METHODS

Audits with a checklist questionnaire

On five different traditional workshop from the south region of Portugal (Alentejo), previously selected in a typology study, were performed a questionnaire. This questionnaire had 65 questions and was divided in two parts. The first part was for Good Hygiene Practices (GHP) evaluation with questions for premises, equipments, hygiene programs, production and process controls. The second part was for check critical control point (CCP) and GHP control points (GHP-CP) and evaluation of the Hygiene program for equipment with sampling on food direct contact surfaces (environmental samples) and final product for hygiene and safety indicators determinations. Different direct contact surfaces on processing area such as knives, tables, walls, cutting, mixing and stuffing machines have been evaluated for *Enterobacteriaceae* counts, as hygiene indicator, and pathogenic flora detection (*Salmonella* spp., *Listeria monocytogenes*, *and Staphylococcus aureus*). The limits criteria for hygiene indicator was Ultra clean≤$2x10^2$ cfu/100cm^2, $3x10^2$ cfu/100cm^2 ≥ Clean ≤ $9x10^2$ cfu/100cm^2 and Not clean ≥ $10x10^2$ cfu/100cm^2. Chouriço were evaluated for pathogenic flora with critical limits such as *Salmonella* spp. absent in 25g, *Listeria monocytogenes* < 100 cfu/g and *Staphylococcus aureus* < 1000 cfu/g.

The total score was 51 and 60 for the first and second part of the questionnaire respectively.

The workshops with a score of 30 in the each part were classified as Sufficient for prerequirements needed for a successful implementation of HACCP (1st part) and validation of prerequirements (2nd part).

Samples collection

The environmental samples were collected in clean and disinfected equipment according to hygiene procedures of each workshop, before the beginning of work. The different direct contact surfaces on processing area were: knives, tables, walls of resting cold room, cutter, mixer and stuffing machines. Approximately an area of 500 cm^2 was sampled from each surface with a sterile cloth dampened with a neutralising solution and according to ISO 18593.

The final product “chouriço” was also collected from each workshop. Five samples were collected from the lot processed in the audit day.

Microbiological determinations

Microbial determinations were carried out to *Enterobacteriaceae* counts in Violet Red Bile agar (VRB agar, Merck, Germany) at 37 ºC for 2 days (ISO 21528-2:2004); Counts were expressed as cfu/100 cm^2.

The preparation of “chouriço” samples for microbial analysis was done in accordance with ISO 6887-1:1999. Occurrence (presence or absence in 25 g) of *Salmonella* and *L. monocytogenes* and enumeration of *Staphylococcus aureus* and *L. monocytogenes* was performed according to the methods described in Talon *et al*. (2007).

Statistical analysis

Statistical data analysis was performed using SPSS 11.5 for Windows.

RESULTS AND DISCUSSION

The results of the check list used in five different traditional workshops to evaluate Good Hygiene Practices (GHP) (premises and procedures) and critical control point (CCP) and GHP control points (GHP-CP) with a supplementary sampling for direct contact surfaces and final product are presented in Figures 1 and 2.).

The data from the checklist conceived to evaluate pre-requirements, GHP and GMP shown that all workshops have been approved by a competent authority. 60% of the workshops had waste treatment but only 20% were performing the selection and separation of solid trash. The water supply was treated in 80% of the workshops. From "Lay Out" evaluation the presence of crossed lines were detected in only 20% of the workshops. The materials used in the construction (walls, floor, ceilings, doors, windows) were appropriate to an easy cleaning in all workshops. Walls and floor material's conservation and its hygiene level were sufficient (40-20%, respectively) to good (60-80%, respectively) in all workshops. All workshops presented windows with fine mesh to keep out insects, electric flytraps, all lights in preparation or processing areas equipped with shields or proper covers, adequate or sufficient ventilation or equipment to minimize odours and vapours and prevent water condensation and washing foot device on the entrance of your workshop. 80% of the establishments were producing in compliance of some Code of Hygiene Good Practices, presenting all sanitary facilities (hand-washing, hand drying devices, toilets) required according to the Codex Alimentarius Commission (2003). Only 40% of the workshops had birds, insects and rats control plan provided by a specialized pest control enterprise. All establishments had equipment and utensils designed and constructed as to be adequately cleanable, and were made with innocuous material that will not cause food contamination.

Regarding the personal hygiene, it was observed that workers had a health certificate at 80% of workshops, but only in 40% they had routine medical examinations. The workers of all workshops used suitable protective clothing (hair nets/caps, boots), changing it in a sufficient to good frequency. At 96.2% of workshops, jewels, watches and other adornments were removed by workers before entering working premises.

The cleaning schedule to floor was daily performed in all workshops while only 20% were doing a daily schedule for walls. 40% of the workshops were Cleaning/disinfection equipment at the end of each use while 60% had a daily frequency. In all workshops practices of removal of solid residues prior to cleaning operations was done, there was a specific equipment used for sterilization of knifes and other utensils and chemicals (pesticides, herbicides, cleaning agents, lubricants, boiler compounds) were stored in concealed designated areas.

From questions related to production and process control, was observed that only pork meat was used in "chouriço" technology, and raw materials and other ingredients were checked on their pre-emption or "best before" date. 80% of the workshops had a sampling analyses control plan.

A regular monitoring on the process's time and temperature was performed in all workshops but only 60% had a plan of metrology calibration control with a specialized enterprise.

This data led to the conclusion that all workshops evaluated have the main pre-requirements. The total score obtained by workshops in the first part of the questionnaire was superior to 30; this was considered Sufficient for prerequirements needed to achieve the possibility to implement the HACCP system successfully.

The workshop with less punctuation on 1st part (34) did not achieve 30 points in the 2nd part due to hygiene indicator high counts and *Listeria monocytogenes* in environmental samples (table and knife), and in final product (Figure 2). Only in workshop 4 was detected the presence of pathogens in equipment surfaces of cutting table and knife. The workshop 5 had a good score on the first part regarding prerequirements for premises, equipment and personal, but revealed a hygiene procedure for equipment not efficient as was possible to conclude by the higher counts of *Enterobacteriaceae* on mixing and stuffing machine and in addition the detection of pathogens (*Listeria monocytogenes* and *St. aureus*) in final product.

From the workshops evaluation using the check list it was possible to observe that several points could be improved to assure product safety, related essentially to the efficiency of equipments hygiene procedures. This check list can be used by small teams existing in traditional workshops to the compilation and registration of data, contributing to the management of safety with the validation of a Hygiene Practices Code. Safety systems should be carefully designed to include validated cleaning procedures, with appropriate documentation to allow for regular audit and review (Malik *et al*., 2003).

It is important to note that WHO (1999) and Codex Alimentarius Commission (2003) permit, when applying HACCP, to be flexible where appropriate, given the context of the application and taking into account the nature and the size of the operation. So for small units the application of a validated Hygiene practices code will be effective to reduce the risk of hazards, being fundamental for subsequent implementation of a HACCP based approach.

The check list seems to be a good tool for safety verification and validation in traditional workshops.

CONCLUSIONS

All the workshops had a score > 30 on the questionnaire 1st part, considered being Sufficient for prerequirements needed to a successful implementation of HACCP. Nevertheless, the workshop with less punctuation on 1st part (34) did not achieve 30 points in the 2nd part due to hygiene indicator high counts and *Listeria monocytogenes* in environmental samples (table and knife), and in final product.

The check list seems to be a good tool for safety verification and validation in traditional workshops.

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Figure 1: Workshops (W1-5) scores obtained in the first part of the check list audit related with prerequirements.

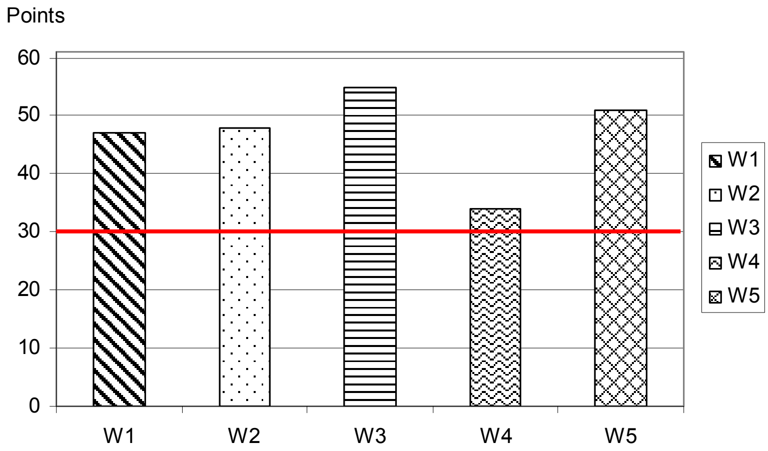
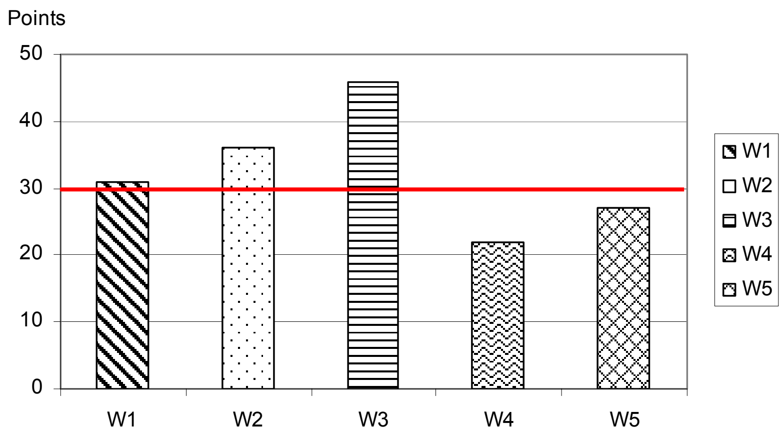


Figure 2: Workshops (W1-5) scores obtained in the second part of the check list audit related with CCP check points and samples microbial analysis.



REPRODUCIBILITY EVALUATION OF CLASSIFICATION BY EXPERT ASSESSORS OF RAW HAM RED SKIN DEFECT

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SUMMARY - The evaluation of qualitative characteristics of fresh pig thighs to be used for seasoning is generally made by expert assessors basing on visual aspect. To estimate quantitatively the degree of objectivity of human evaluation of visual aspect, digital images were acquired on a series of raw ham samples and then subjected to the classification by expert assessors in three quality categories related to the red skin defect. The results obtained from the panel have been analysed in terms of Validity (correctness of the response) and Reliability (ability of the assessor to reproduce his own results). Moreover, also the percentage of assignations of each sample to the correct class was estimated. The results suggest that the proposed approach can be used both to monitor the reliability of the single assessors, and to identify samples whose class assignation is reasonably indubitable.

Key Words: Raw ham, Visual aspect, Red skin defect, Digital RGB images

INTRODUCTION

The evaluation of aspect-related characteristics of fresh pig thighs destined to seasoning is becoming a key aspect in the production of PDO ham. In fact, a preliminary classification of fresh pig thighs on the basis of the presence/absence and of the extent of defects, which could influence the quality of the seasoned product, is of key importance both by the logistic and by the financial points of view.

At present, estimation of raw hams is based on weight measurements and on the evaluation of visual aspect, made by expert assessors. However, this kind of evaluation is subjected to a series of drawbacks, mainly connected to its subjectivity and - sometimes - to the inconsistency of the human eye, but also to the dependency on available specialised manpower. Moreover, the human visual evaluation is operator dependent, and therefore not easily transferable between different production lines and/or industries. In this context, automated systems capable of acquiring and elaborating aspect-related data are surely valuable tools, since they can furnish objective, reproducible and transferable information about the appearance of the analysed products (Lo Fiego *et al.,* 2007).

In the assessment of food colour, it is common practice to use spectrophotometers or light sensitive cells (such as photodiodes or photomultipliers) as light sensors to quantify reflectance, transmittance or absorbance characteristics. The most diffused instruments involved in these measurements evaluate only restricted areas around the analysed food sample (spot colorimeters), thus being not appropriate for food products having an inhomogeneous aspect like raw hams, or estimate the overall light reflectance from the entire surface of a given portion of the food matrix (integrating spheres), giving only a global colour evaluation without any information about its local variability, and being not easy to implement on-line.

On the other hand, recent advances in image acquisition technology offer the possibility to use technically sophisticated apparatuses accessible at relatively low costs, which are able to evaluate up to millions of pixels of the analysed food product, allowing the analysis of light reflectance both locally and globally. The common digital cameras, at variance with the traditional light sensors, offer also the possibility of performing a detailed evaluation of food products with inhomogeneous colour, since every different colour present in the image of the analysed food matrix can be accounted for by one or more pixels (Antonelli *et al.,* 2004; Ulrici *et al.,* 2005).

For these reasons, in the last 15 years the analysis of digital images by proper mathematical tools has gained an increasing interest in industrial applications in general, and in the field of food analysis in particular (Geladi and Grahn, 1996; Panigrahi and Gunasekaran, 2001).

However, the digital-image-based automated methods used for the at- or on-line assessment of the quality of raw materials, intermediate or finite products are generally based on mathematical models which somehow relate the information extracted from the image data to some reference measurements, frequently deriving from the human evaluation. In other terms, the information acquired by the digital image can be used to attribute an unknown sample to a given class or to predict the value of a given parameter only after that in some manner (i.e., using a given algorithm) the automated system has "learned" from the human experience what class or what parameter value correspond to each one of a series of samples used for training the system. Thus, if the reference measurements deriving from human evaluations are affected by large errors, the automated system will be also affected by errors at least as large as the reference measurements.

Therefore, in view of the construction of digital-image-based automated systems (e.g. for the classification of food products with inhomogeneous aspect like raw hams), the definition of strategies aimed at obtaining as much as possible objective estimates of the visual characteristics of interest for the analysed products is mandatory. In fact, a quantitative estimate of the reproducibility of the evaluations made by a panel of expert assessors can be used for the selection of the samples to be used for the construction of the dataset to train the automated system, and allows also to evaluate its performance (Foca *et al.*, 2007). Moreover, also the reliability of the assessors belonging to the panel can be estimated, thus allowing to select only the best ones for subsequent panel sessions, and/or to furnish a further training to those assessors giving less reproducible evaluations.

Basing on these considerations, in the present work digital RGB images were acquired on a series of raw ham samples and then, by means of a graphical user interface implemented *ad hoc*, subjected repeatedly in random order to the classification by a panel of six expert assessors in three quality categories related to the red skin defect. The results obtained from the panel have been analysed by proper statistical tools on the basis of two performance indicators of the assessors: Validity, i.e., the correctness of the response, and Reliability, i.e., the ability of the assessor to reproduce his own results. Moreover, also the percentage of assignations of each sample to the correct class was estimated, in order to identify samples whose class assignation is reasonably indubitable, e.g. to be used for the development, in the next future, of automated classification methods.

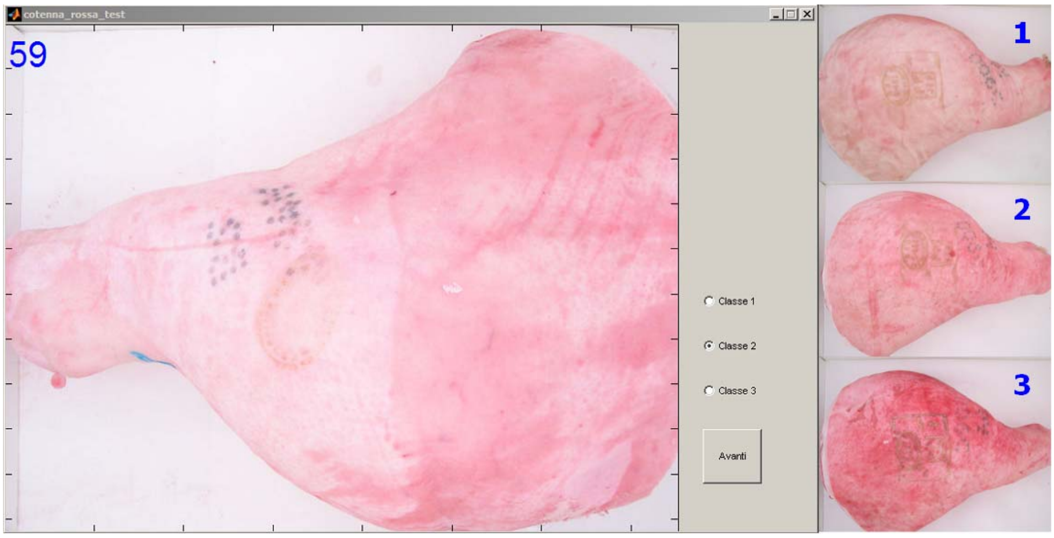
MATERIAL AND METHODS

For this study, 198 left thighs destined to the Parma ham production, coming from heavy pigs slaughtered in one plant during 5 different days, were used. After 24 hours of cooling at 0 - 4°C, on each thigh digital images of both the external and internal surface of the thighs were acquired by means of a Nikon Coolpix 5400 digital camera equipped with a lens with 5.8-24 mm focal length (Nikon corp., Tokyo, Japan). In the present study only the image of the external thigh surface was considered. With the aim of having as much as possible constant and homogeneous lighting conditions, the camera was mounted on a white painted wooden box equipped with 8×25 W tungsten lamps, where each thigh was put into in order to be photographed. Digital images were taken in JPEG format with a spatial resolution of 2592×1924 pixels, using white balance, with a 1/125 s shutter speed and an f/5.6 lens aperture.

Six assessors were asked to classify individually the images in 3 classes related to the red skin defect, namely class 1 for raw ham samples without red skin defect, class 2 for samples with intermediate defects and class 3 for samples with severe defects. For each one of the 198 digital images two evaluations have been performed by each assessor; to this aim, firstly all the 198 images were evaluated sequentially, and then the replicated evaluations have been performed by sorting the images in random order. For all the assessors the overall sequence of 396 images was the same.

Assessments have been made by means of a graphical user interface implemented *ad hoc* in the Matlab ver. 7.0 environment (MathWorks Inc., Natick, MA, USA), which allowed the assessors to easily evaluate the digital image series, also using reference images for the three quality classes, as reported in Fig. 1. The software showed in sequence each one of the 396 images to be evaluated (whose progressive number was reported in the upper left corner of the image) and required that the proper class was assigned by the assessor before he passed to the next image. The program does not allow a user to return to previously evaluated samples, so that the assessor was constrained to consider each sample independently. Moreover, in order to avoid inconsistent evaluations due to weariness of the assessors, the software allows to exit at any time during the evaluation simply by clicking the “close” button and to restart from where it was interrupted, simply specifying the number of the last evaluated sample.

Figure 1. Graphical user interface used by the assessors for the panel test (left side), together with the reference images of the three quality classes (right side, with the respective class numbers specified in the upper right corner).



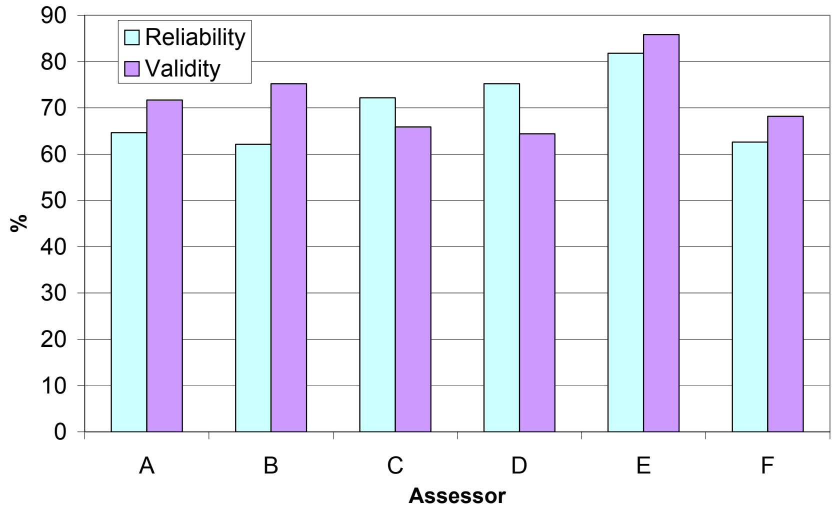
The results of the class assignations of the samples by the assessors were expressed in terms of assessors Validity (V) and Reliability (R). In particular, we defined the assessor Validity as the percentage of assignations to the correct class (estimating the correctness of the assessor response, i.e. his agreement with the whole panel) and the assessor Reliability as the percentage of assignations to the same class over the two repeated evaluations of each sample (independent of the correct class), which gives an estimation of the ability of each individual assessor to reproduce his results. Moreover, the Global Performance of each assessor was defined as the sum of his Validity and Reliability scores, and used to evaluate the uniformity of the panel by a chi-squared test.

In addition, for each sample we defined the % Overall Agreement on Samples Attributions (%OASA) as the percentage of assignations of each sample to the correct class, i.e., the number of times that every single sample has been assigned to the correct class, divided by the total number of assignations for that sample (= number of assessors × number of replicate assignations by each assessor), and multiplied by 100. Since the definition of “correct” class for a given sample is not an absolute concept and is not known a priori (i.e., before the evaluation of the samples aspect by means of the digital images and the subsequent assignation by the assessors themselves), this was the main matter that had to be confronted at this step of the work. This information was fundamental for the determination of the Validity of the assignation by the assessors and of the %OASA of each sample. Therefore, we decided to unconditionally assign each sample to the most frequently selected class (that we called correct), i.e., to the mode of the 12 class assignation values for each sample. Moreover, the dataset consisting of the 12 evaluations (2 replicate evaluations for each one of the 6 assessors) of the 198 samples was also analysed by Principal Component Analysis (PCA) (Wold *et al.*, 1987), in order to estimate the degree of separation of the quality classes in the multivariate space of the various evaluations, also in view of the contribution by the various assessors. All calculations were performed by means of Matlab ver. 7.0 (MathWorks Inc., Natick, MA, USA) and of PLS Toolbox ver. 4.0 (Eigenvector Research Inc., WA, USA).

RESULTS AND DISCUSSION

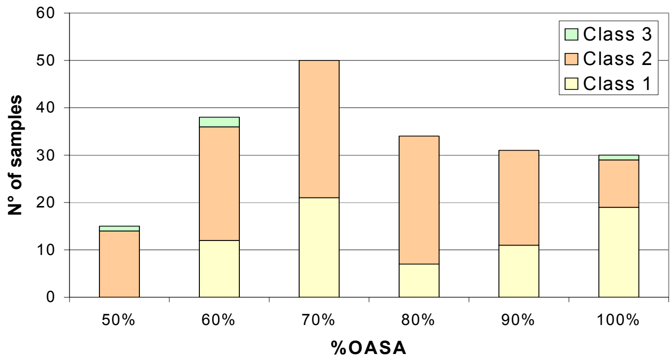
The distribution of the Validity and Reliability values of each assessor (indicated with letters from A to F), computed from the results of the panel test is reported in Fig. 2. It can be observed that the values of V and R show comparable values, in the sense that the difference between replicated estimations from a single assessor is comparable to the difference between the evaluations of different assessors. This observation was confirmed by a one-tailed *t*-test on paired data, performed in order to compare the V values of each assessor with the corresponding R values. The calculated *t* value was equal to 0.58 and led to the acceptance of the null hypothesis (i.e., equivalence of Validity and Reliability) at the 95% probability level (t_{CRIT} = 2.01).

Figure 2. Validity and Reliability values for each assessor.



Then, the uniformity of the panel was also tested, in order to verify the possible presence of assessors whose performance is significantly different, in particular to evaluate if the performance of assessor E is significantly better than the other assessors performance. To this aim, the Global Performance of each assessor was defined as the sum of his V and R values, and its distribution was compared by a chi-squared test to the corresponding uniform distribution. At the 95% probability level (χ^2_{CRIT}= 11.07) the test confirmed the uniformity of the performance of the six assessors (χ^2= 6.05). For what the raw ham images are concerned, Fig. 3 shows the distribution histograms of the %OASA values separately for the three classes.

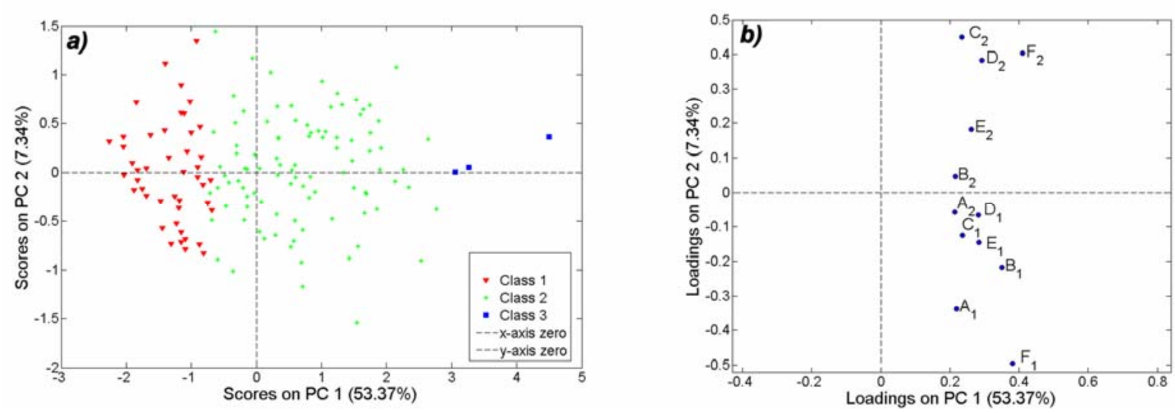
Fig. 3. Distribution histograms of % Overall Agreement on Samples Attributions; the different colours refer to the different quality classes.



First of all, it can be observed that only 4 samples have been assigned to class 3, i.e. classified as raw hams with severe defects, while the other two classes are much larger (70 samples in class 1 and 124 samples in class 2). On the whole, there are not significant differences in the %OASA values for the three classes even if class 1 shows on the average a better level of agreement. The minimum %OASA value (obtained for 14 samples of class 2 and 1 sample of class 3) is equal to 50%, indicating that at least 6 evaluations out of 12 converged to the same class value. The greater part of samples have %OASA values around 70%, and only for 30 out of the 198 samples a perfect agreement (i.e., 100 %OASA) among the 12 different evaluations was obtained. On the whole, only 95 samples out of 198 obtained %OASA values greater than 70%, thus indicating that for a very high number of samples the level of agreement among the assessors is rather low.

The results of the panel have been also evaluated by means of PCA (Fig. 4). The scores of the first 2 PCs are reported in Fig.4a, where the samples have been represented with different symbols depending on their correct class, as it was previously defined in the MATERIAL AND METHODS section. It can be observed that the class distinction is accounted for by the first PC, while PC2 reflects the differences between the assessors (the further PCs also reflecting these differences – results not reported for conciseness reasons). In particular, the corresponding loadings plots (Fig. 4b) indicate that PC2 mainly reflects the differences between the two replicate estimates of the assessors.

Figure 4. First two PCs obtained by PCA performed on the matrix of the evaluations: a) scores plot, with different symbols indicating different classes; and b) loadings plot, with different letters indicating different assessors and numbers indicating the repeated evaluations.



CONCLUSIONS

In the present paper, an attempt has been made to estimate the degree of reproducibility of the visual evaluation method used for quality classification of raw ham basing on the red skin defect.

The assessors were judged in terms of Validity (reflecting their general agreement) and Reliability (reflecting the ability of the single to reproduce his results), whose comparison demonstrated that, on the whole, the variability in the repeated evaluations of a single assessor is comparable to that of evaluations from different assessors. These results suggest that the proposed approach can be used to monitor the reliability of single assessors in order to check, over the time, the constant quality of their performance.

In addition, the evaluation of %OASA values allows to identify samples whose class assignation is certain, which could be used as reference samples for the development of robust automated classification methods. In fact, this information could be particularly useful for the purpose of creating a reliable and robust automated classification method of raw ham red skin defect, on the basis of RGB images.

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ANALYSIS OF COAT COLOUR GENES FOR TRACEABILITY OF CINTA SENESE PRODUCTS

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SUMMARY - Cinta Senese is a white belted pig breed native of Central Italy. Market prices of Cinta Senese (CS) of fresh and cured products are very high and this is due to the quality of the raw material. Assurance that products are made using only CS individuals is what consumers want. Therefore the institution of a protocol for a DNA-based traceability of Cinta Senese products is desirable. Coat colour genes could allow to distinguish this belted breed from the most widespread breeds reared in Italy. Four polymorphisms at locus MC1R and one at locus KIT were analyzed in 180 Cinta Senese (CS) purebred, 18 F1 Large White x CS and 15 F1 Duroc x CS individuals. Cinta Senese is homozygous i/i at locus KIT, whereas it shows two allelic forms at locus MC1R: MC1R*6 and MC1R*3. The results confirm that it is possible to distinguish Cinta Senese from both white and red pig breeds and even from crossbred animals. The suggested protocol might consist in a PCR test genotyping KIT locus combined with at least one of the RFLPs test at the MC1R locus. Furthermore, sequencing of the PCR products were performed in order to validate the protocol.

Key words: RFLPs, traceability, KIT, MC1R, Cinta Senese

INTRODUCTION

Cinta Senese is a white belted pig breed native of Italy. This breed is characterized by a high quality meat whereas its productive and reproductive performances are poor. The breed is currently used to produce fresh and cured products. Prices of Cinta Senese products are still too high for a market basically founded on trust among breeders, producers and consumers. Coat colour genes could allow to discriminate this belted breed from the most widespread breeds reared in Italy. Two loci, *Extension (MC1R gene)* and *Dominant White (KIT gene)* have a major influence on coat colour. The alleles were described and their association with breeds and colours was determined (Giuffra *et al.,* 2002; Johansson Moller *et al.*, 1996; Kijas *et al.*, 1998, 2001; Marklund *et al.*, 1998; Pielberg *et al.*, 2002). The institution of a protocol for a DNA-based traceability of Cinta Senese products is desirable. With the aim to identify molecular markers that could be used for the traceability and valorisation of the products obtained from this breed we investigated polymorphisms in two genes affecting coat colour, *MC1R* and *KIT* coded by the extension and dominant white/white belted loci, respectively.

MATERIAL AND METHODS

DNA samples were obtained from hair, blood and freeze-dried meat of 180 Cinta Senese animals and several commercial breeds were used as control (table1). Simple tests were developed for both *MC1R* and *KIT* genes (Kijas *et al.*, 1998, 2001; Marklund *et al.*, 1998; Pielberg *et al.*, 2002). Four polymorphisms were analysed for the *MC1R* gene. These consisted of three RFLPs (MC1R-124, MC1R-243 I, MC1R-243 II) as well as a two base pair insertion (+CC) at the 5' end of the coding sequence (MC1R-23) (Kijas *et al.*, 1998, 2001). The presence of KIT duplication was detected by a run of a PCR test (Giuffra *et al.*, 2002). Amplification conditions and fragments size are summarized in table 2.

Results of RFLPs analyses and PCR test were visualized on a 7% polyacrilamide gel, while the detection of the two base pair insertion was obtained with an ABI Prism sequencer.

Table 1. Breeds and number of individuals per locus investigated.

Breed	Locus			
	MC1R-23	MC1R-124	MC1R-243	KIT
Cinta Senese	36	180	180	171
Large White	2	14	14	14
Landrace	---	5	5	5
Pietrain	3	5	5	5
Duroc	---	10	10	---
Hampshire	5	---	---	---
Large White x Cinta Senese	---	18	18	18
Duroc x Cinta Senese	---	15	15	15

Table 2. Amplification conditions and fragments size

Amplification mix			Cycling conditions				
Reagent	Concentration	Volume µl	Temperature		Locus	Annealing temperature	Fragment size
H_2O		12.0	95°C	5'	MC1R 23	58°C	232bp 234bp
Taq buffer	10 X	2.0	35 cycles				
$MgCl_2$	50 nM	1.0	95°C	30''	MC1R 124	65°C	196bp
Primer For	10 pmol/ µl	1.0	Ann. Temp.	30''			
Primer Rev	10 pmol/ µl	1.0	72°C	30''	MC1R 243	64°C	154bp
dNTP's	10mM	0.8					
Taq	5U/ µl	0.2	72°C	5'	KIT	66°C	152bp
DNA		2.0					
Total		20.0					

RESULTS AND DISCUSSION

All Cinta Senese animals resulted fixed for the absence of the gene duplication at KIT locus, while at MC1R locus some differences among animals appeared.The three PCR-RFLP analyses produced two fragments for all 180 Cinta Senese individuals; the +CC insertion was detected in the 0.14 per cent of the cases.

Table 3: Results of analyses at MC1R and KIT loci and the association among alleles and breeds.

Breed	Allele	Locus				Allele	Locus
		MC1R-23	MC1R 124	MC1R 243 I	MC1R 243 II		KIT dup.
Large White	E^P	+CC/+CC	+/+	+/+	+/+	I/I [(2)]	YES
Landrace	E^P	+CC/+CC	+/+	+/+	+/+	I/I [(2)]	YES
Pietrain	E^P	+CC/+CC	+/+	+/+	+/+	I^P/I^P [(2)]	YES
Duroc	E	-CC/-CC	-/-	-/-	-/-	i/i	NO
Cinta Senese	E^{D2} /(E^P)*	-CC/+CC	+/+	+/+	+/+	i/i	NO
Duroc x CS	**	**	+/-	+/-	+/-	i/i	NO
Large White x CS	**	**	+/+	+/+	+/+	I/i	YES
Hampshire[(1)]	E^{D2}	-CC/-CC	+/+	+/+	+/+	i/i	YES

[(1)] Kijas et al. (1998) [(2)] Giuffra et al. (2002)
* Allele frequency: E^{D2} 0.86 and E^P 0.14 ** Analysis not performed
+/+ Homozygous for enzyme cut; -/- Homozygous for cut absence; +/- Heterozygous
+CC presence of two base pair insertion; -CC absence of two base pair insertion

The comparative analysis amongst all genotyped breeds showed that Cinta Senese has an almost unique output combination. The absence of the KIT duplication allows to discriminate Cinta Senese from the most important white breeds reared in Italy, whereas PCR-RFLP of MC1R-124 provides a powerful tool to distinguish Cinta Senese from Duroc. With these analyses is also possible to detect the hybrids obtained using Large White or Duroc on Cinta Senese pigs.

CONCLUSIONS

Considering the results obtained in the present study it is possible to outline a traceability marker-based protocol. It might consist of the PCR test genotyping KIT locus combined with at least one of the RFLPs test at the MC1R gene.

The procedure could be used to settle legal issues or, considering the simplicity of the analyses and the limited number of animals involved, to systematically control all the Cinta Senese products. This latter solution could allow to establish a genetically guaranteed brand. Nevertheless the commercial use of these analyses requires the preliminary check of patent rights. Furthermore it has to be pointed out that the protocol will not be able to fully distinguish Cinta Senese breed from the other belted breeds distributed all around the world. Currently Hampshire breed is the sole belted breed reared in Italy but it is impossible to exclude the possibility of an introduction of other similar coat colour breeds in the future.

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UTILIZATION OF NIR SPECTRAL DATA AND MULTIVARIATE MODELS FOR IBERIAN PIG CARCASSES GRADING

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SUMMARY –. Previous research has demonstrated the ability of Near Infrared Spectroscopy (NIRS) to accurately grading Iberian pig carcasses according to the feeding regime, by using calibration equations for the prediction of the major fatty acids of the adipose tissue. The objective of this paper was to develop and evaluate NIRS predictive models for carcass grading, based on the (NIRS) data “per se”. Principal Component Analysis (PCA) was applied on the NIR spectral data obtained, from the adipose tissue of pigs belonging to three different commercial categories *(bellota, recebo and cebo)* The spectral data of a total of 470 samples from pigs reared , between 2001 and 2004, were obtained by using a fiber optic probe. The multivariate classification model was developed with the WINISI software. The PCA model was established using data from the three commercial categories, that is, *Bellota* (feed with acorns and grass, n=60), *Recebo* (combination of acorns, grass and commercial feed, n=60) and *Cebo* (commercial feed only, n= 60). Model validation, performed using samples not included in the model, correctly graded 100% of the animals fed on commercial feed and only a 1.4% of the animals belonging to the two other feeding categories were erroneously graded.

Key Words*:* Iberian pig, carcass grading, NIR Spectroscopy, fiber optic probe

INTRODUCTION

The consumption of fresh meat and cured products of the Iberian pig has undergone a significant increase in the last few years. The quality of its products depends on the quantity of acorns and grass pasture consumed in the final stages of fattening period. However, the production of acorns is limited and so top quality products reach higher prices on the market. This compels the carrying out of controls on the animals to guarantee quality of the products obtained from them.

The Spanish parliamentary act (Real Decreto) 1083/2001 established the requirements that pigs should fulfil established requirements to be classified as “Bellota”, “Recebo” and “Cebo”, according to their type of feeding. “Bellota” animals are those that have only consumed acorns and grass pasture in the final fattening stages. The “Recebo” pigs complete their fattening based on fodder after having reached a minimum weight of 28,5kg through the consumption of acorns and pasture. “Cebo” quality types are those that do not fulfil the minimum “Recebo” quality required and they have only consumed commercial feed.

Although weights at the beginning and at the end of the fattening period as well as the weight increase due to the consumption of acorns and pasture and the minimum amount of control visits to be made are indicated in the Decreto, the verification of these requirements is difficult to carry out (Garcia Olmo and De Pedro, 2002). For this reason, and given the influence of the feeding in the fat composition (De Pedro and Secondi, 1991; Lopez-Bote 1998; Cava et al., 2000; De Pedro, 2001), analytic techniques have been searched to identify and differentiate the type of diet that these animals have consumed in order to classify accordingly their products, especially the most expensive ones (ham, shoulder and loin).

Gas chromatography is the most used technique to determine the content of the subcutaneous fat in pigs in the principle fatty acids and is officially recognized Howevwe, the use of certain raw materials in the formulation of commercial feed enables obtaining fatty acid profiles in the Iberian pig fat similar to those reached by animals fed exclusively with acorn and pasture. This has motivated the search for other differentiating diet parameters such as tocopherols, hydrocarbures, phospholipids, isotopes, etc. (Cava et al., 2000; Tejeda et al., 2001; Delgado et al., 2004; Gomero-Pasadas et al., 2006).

Previous studies carried out in the Animal Production Department of the University of Cordoba have shown NIRS technique is feasible not only to predict the fatty acids composition of Iberian pig fat, but also to classify carcasses from that composition or from its spectral information (Hervás et al., 1994; De Pedro et al., 1995, 2004; García Olmo, 2002, García Olmo et al., 2001).

The objective of this study was to develop and evaluate NIRS prediction models for caracass grading, based on NIRS methodology "per se" spectral data obtained directly from adipose tissue samples of Iberian pigs.

MATERIAL AND METHODS

The present work was carried out with 470 Iberian pigs between 2001 and 2004 (399 fattened on the "montanera" (Bellota or Recebo) and 71 with commercial feed only). A sample of fat was taken in accordance with the official procedures (ORDEN PRE/3844/2004), before the carcass breakdown.

NIR data samples were recorded using a remote reflectance fiber optic probe (NR-6539-A) connected to a Foss-NIRSystems 6500 spectrophotometer (Foss NIRSystems, Silver Springs, MD, USA) equipped with an autogain detector. The spectra were collected, between 400 and 2200 nm at 2 nm intervals, with WINISI software ver. 1.5 (Infrasoft International, Port Matilda, PA, USA). This software was used for the chemometric analysis of NIR data, to develop of model the principal component analysis (PCA).

The Principle Components model (PCA) was established using the spectral data of samples corresponding to three commercial categories, that is, *Bellota* (n=60), *Recebo* (n=60) and *Cebo* (n=60). The validation of the model, with samples not included in the modelo, was carried out using animals belonging to groups classified (according to the available field information) as "Bellota" (n=206), "Recebo" (n=73) and "Cebo" (n=11) category. The determination of the fatty acids content by gas chromatography of liquid fat samples carried out in the Laboratorio Agroalimentario of Junta de Andalucía, in Córdoba. The methyl esters of fatty acids were extracted with hexane and were determined, using a Perkin-Elmer Sigma 3D with FID detector.

RESULTS

Table 1 shows the results of the classification model (number and percentage) of the samples used to develop the classification model of Iberian pig carcasses are presented in Table 1.

Table 1 Classification (number and percentage) of the samples used to develop the classification model of Iberian pig carcasses.

ORIGINAL CATEGORY		CLASSIFIED BY			TOTAL
		BELLOTA	RECEBO	CEBO	
BELLOTA	nº	45	15	0	60
	%	75,0	25,0	0	100
RECEBO	nº	24	31	5	60
	%	40,0	51,7	8,3	100
CEBO	nº	0	0	60	60
	%	0	0	100	100

All the samples from the “Cebo” group were classified as such, which indicates that the spectral characteristics of these samples were clearly different to those in the other two categories and, therefore, have a different physicochemical composition. Moreover, no sample from the Bellota group was classified as “Cebo”. However, 25% of samples from the Bellota group were classified as “Recebo” . This could be, in part, due to errors of the model or even due to the fact that these samples classified as “Recebo” were from animals which consumed limited amounts of acorns and less grass, and presented a physicochemical composition more similar to that animals in the “Recebo” group, where the consumption of this type of diet is relatively lower. This variability in the diet became more apparent in the “Recebo” group, where 8.3 % of samples were classified as “Cebo”, and 40 % as “Bellota”. Given the fact that the quality standards (weights at the beginning and at the end of the “montanera”) are applied globally by groups and not individually to each animal within the groups, these results seem to be quite logical. It is possible to have in a given group animal that have eaten important amounts of acorns and therefore have characteristics more similar to those of “Bellota” . On the other hand if the a high consumption of commercial feed, once the diet based on acorns has finished, could produce carcasses more like the “Cebo” type.

In Table 2 the results of applying the classification of Iberian pig carcasses to the validation group are presented.

Table 2. Classification (number and percentage) of the samples belonging to the validation group according to the model of Iberian pig carcasses developed.

ORIGINAL CATEGORY		CLASSIFIED BY			TOTAL
		BELLOTA	RECEBO	CEBO	
BELLOTA	nº	138	68	0	206
	%	67,0	33,0	0	100
RECEBO	nº	19	50	4	73
	%	26,0	68,5	5	100
CEBO	nº	0	0	11	11
	%	0,0	0,0	100,0	100

The results of the classification of validation samples of the model (table 2) are similar to those obtained in the development of the model (Table 1). No samples of groups with a diet consisting exclusively of acorns and grass were classified in the “Cebo” group and none of those fed with commercial feed were graded as “Recebo” or “Bellota” . The variability of the animals fattened on the “montanera” was still apparent, as not all the animals of a group were of the same quality. Finally, it should be mentioned that only 4 of the 73 (5%) animals fattened on “montanera” with a complement of commercial feed (“Recebo”) were classified as “Cebo” . This supposes that 1.43 % of the total of animals fattened on “montanera” (279) have typical “Cebo” characteristics.

The results obtained both in the development and the validation of the model of classification indicated that the NIRS methodolgy, with the spectral information “per se” of the subcutaneous fat samples, allows to establish differences among animals which have recieved different diets and, consequently to individually.classify animals within each group That will allow a much better quality classification of the products obtained.

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MITOCHONDRIAL DNA HAPLOTYPING IN PORTUGUESE PIG BREEDS AND APPLICATIONS IN THE TRACEABILITY OF PROCESSED PRODUCTS

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Summary - Three local breeds of domestic pig (*Sus scrofa*) are officially recognized in Portugal (Alentejano, Bísaro and Malhado de Alcobaça) and have regained market interest for high quality local and traditional products that would benefit from certification of origin. We present the mitochondrial haplotype characterization of the extant populations as a pre-requisite for the establishment of the basis of a diagnostic system for certification of genetic origin and traceability. A total of 136 non-related individuals assigned to the three breeds were sampled and surveyed by sequencing. These sequences were analyzed for probability of assignment against published sequences of Landrace, Large White, Duroc and Pietrain. This preliminary picture of maternal lineages already shows distinct breed genetic profiles, with shared and exclusive haplotypes and their frequencies among breeds. A preliminary model that shows the probability of assignment of a given haploytype to a breed is herein presented.

Key words: *Sus scrofa, mitochondrial DNA, haplotyping, traceability*

INTRODUCTION

Three autochthonous breeds of domestic pig (*Sus scrofa*) are officially recognized in Portugal: Alentejano, Bísaro and Malhado de Alcobaça, geographically distributed in the Southeast, North and Centre of the country, respectively. After a period of neglect that followed the general trend for local breeds in many parts of the world, these breeds have recently regained attention and appraisal for their meat and derived processed products. The present work characterizes the diversity of the female lineages present in these breeds based on the comparative sequence analysis of a hypervariable fragment of mitochondrial DNA (mtDNA) control region with published sequence data on the exotic breeds Landrace, Large White, Duroc and Pietrain. As mtDNA is often the only genetic material reliably extractable from processed products, the characterization of the extant populations is a pre-requisite for the establishment of the basis of a diagnostic system for certification of genetic origin and traceability. This genetic marker is still in general poorly studied in Portuguese pig breeds and little data was published so far. In this work we aimed at preliminarily presenting a picture of mitochondrial lineages in Portuguese pig breeds and at assessing the potential of mitochondrial haplotyping for the traceability of processed products back to the population of origin at the breed level.

MATERIAL AND METHODS

A total of 136 non-related individuals assigned to Portuguese breeds Alentejano, Bísaro and Malhado de Alcobaça were sampled throughout 23 farms and a 611 bp segment of the mtDNA control region between positions 16561 and 610 was surveyed by sequencing. Primer pair used was F-5'-ACTAACTCCGCCATCAGCAC-3' and R-5'-CTGTGTTAGGGCCTTTGACG-3'. The sequences obtained were compared by alignment to a reference haplotype (Lin *et al*., 1999). The analysis includes previously published sequences (Fang and Andersson, 2006) on exotic breeds. The probability of assignment of a haplotype belonging to a given breed against another was calculated as follows:

$$P = \frac{f_A / f_B}{[(f_A / f_B) + 1]}$$

with f_A - haplotype frequency in breed A and f_B - haplotype frequency in breed B

RESULTS AND DISCUSSION

Sequences obtained for Bísaro (n=86), Malhado de Alcobaça (n=34) and Alentejano (n=16) and sequences publicly available for Duroc (n=9), Landrace (n=36), Pietrain (n=8) and Large White (n=28) were grouped according to identity ignoring indels (Table 1). Haplotypes were named arbitrarily since no standardized nomenclature system is currently available. Portuguese pig breeds show some private (not shared) mitochondrial haplotypes when compared to exotic Landrace, White Large, Pietrain and Duroc, namely S3 and S4 in Malhado de Alcobaça, S7, S9 and S10 in Alentejano, and S12 and 13 in Bísaro. These are, consequently, the most informative haplotypes for breed traceability purposes. Although these results reflect, at least with regards to Duroc, Pietrain and Alentejano, some degree of sampling bias, since the number of individuals tested is low, for the purpose of developing a preliminary model of mtDNA haplotyping for application in traceability, it is assumed that these breeds' profiles are complete. The assignment probabilities were calculated for Alentejano against Duroc (Table 2), and Bísaro against Landrace, Large White, Pietrain and Duroc (Table 3). These pairs were selected for testing because they are the mixture events more likely to occur, whether by crossbreeding or in product mislabelling. Four Alentejano haplotypes (S1, S7, S9, S10; 80%) allow 100% assignment probability against Duroc, whereas one haplotype (S6) is shared between the breeds and, consequently, not informative. The same probability of assignment analysis for haplotypes present in the Bísaro breed against the other breeds shows that private haplotypes S12 and S13 are the most informative and allow for 100% discrimination against the four breeds Landrace, Large White, Pietrain and Duroc. Haplotype S2 is 100% informative against three breeds (Large White, Pietrain and Duroc). Haplotype S8 is also 100% informative against three breeds (Landrace, Pietrain and Duroc). S11 is 100% informative against Pietrain and Duroc. S5 is the only haplotype shared among all breeds and, therefore, not informative.

Table 1. Mitochondrial haplotypes and frequencies as observed in local and exotic pig breeds. Private haplotypes are highlighted.

	Bísaro	Malhado	Alentejano	Duroc	Landrace	Large White	Pietrain
S1	0	0,382	0,063	0	0,167	0	0
S2	0,128	0,412	0	0	0,028	0	0
S3	0	0,118	0	0	0	0	0
S4	0	0,147	0	0	0	0	0
S5	0,442	0	0	0,667	0,194	0,071	0,250
S6	0,116	0	0,125	0,333	0,083	0	0
S7	0	0	0,375	0	0	0	0
S8	0,140	0	0	0	0	0,036	0
S9	0	0	0,188	0	0	0	0
S10	0	0	0,063	0	0	0	0
S11	0,058	0	0	0	0,056	0,143	0
S12	0,012	0	0	0	0	0	0
S13	0,105	0	0	0	0	0	0
hap13	0	0	0	0	0,028	0,214	0
hap16	0	0	0	0	0,056	0,286	0
hap22	0	0	0	0	0,056	0	0
hap23	0	0	0	0	0,056	0	0
hap24	0	0	0	0	0,056	0	0
hap25	0	0	0	0	0,056	0	0
hap26	0	0	0	0	0,028	0	0
hap27	0	0	0	0	0,056	0	0
hap28	0	0	0	0	0,083	0	0
hap29	0	0	0	0	0	0,036	0
hap30	0	0	0	0	0	0,143	0
hap31	0	0	0	0	0	0,036	0
hap32	0	0	0	0	0	0,036	0
hap33	0	0	0	0	0	0	0,250
hap34	0	0	0	0	0	0	0,500

Table 2. Relative assignment probabilities (%) of Alentejano haplotypes against Duroc haplotypes.

Alentejano	Duroc
S1	100
S6	27,27
S7	100
S9	100
S10	100

Table 3. Relative assignment probabilities (%) of Bísaro haplotypes against Landrace, Large White, Pietrain and Duroc haplotypes.

Bísaro	Landrace	Large White	Pietrain	Duroc
S2	82,16	100	100	100
S5	69,44	86,08	63,87	39,86
S6	58,25	100	100	25,86
S8	100	79,62	100	100
S11	51,14	28,93	100	100
S12	100	100	100	100
S13	100	100	100	100

CONCLUSIONS

This preliminary survey shows that diagnostic assays based in mtDNA polymorphisms that will discriminate the population of origin of processed products can be developed, with varying and case-sensitive probabilities of discrimination.

Future developments will include further analysis of haplotype composition of the Alentejano breed comparing to other Southern Iberian breeds, as well as further mtDNA analysis in exotic breeds that putatively replace local breeds in crossings and processed products. To some extent, it will also allow for the assessment of the crossbreeding extension of local breeds with non-local animals. Provided extensive data are compiled and used along with other criteria, this research can contribute to the establishment of the genetic profiles of breeds, genetic diversity assessment and comparisons with other populations.

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SESSION 6
SOCIO-ECONOMICAL DYNAMICS FOR TYPICAL MEAT PRODUCTS

Consumer Behaviour: the decision-making process relating to meat choices

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Summary - Food comes in infinite variety, and food choices are a major component of all purchase decisions made by consumers (Grunert, 1997). However, in spite of the research that has been done, mainly in the last two decades, there is no singular commonly accepted model for consumer behaviour relating to food choice. In this article, a discussion of the most widely accepted stages in consumers' decision-making process will be presented, emphasizing choice of meat products in the Mediterranean context. In general, meat choice and consumption are based on a cognitive decision-making process and take account of external stimuli surrounding that choice and consumption. Past experience, sensory perception, and emotion or affect are important influences, in particular for food products but, at some point of the experience with the product, an evaluation based on some criteria (objective or not) is made by the consumers of that product. Depending on the product and on the situation, the complexity of the choice may vary but, usually, there is a problem-solving approach to choice, even if affect or less rational factors influence the way people solve that problem.

Key words: Consumer, behaviour, choices, meat products

PERSPECTIVES ON FOOD DECISION-MAKING

Every day consumers make decisions about food products that vary greatly in their degree of complexity. Some of these decisions are made routinely and automatically, while others, more important to them, are more reflective and imply a higher level of involvement and, consequently, more information processing and effort. Some can clearly be viewed as rational choices, while others are, apparently, irrational. Therefore, according to Solomon *et al.* (2002), consumers decision-making may range from actions based on habits and routines that individuals develop over time, to novel situations of consumption, involving a great deal of risk, where consumers must carefully collect and analyse information prior to making a choice.

Specific models of consumer behaviour with respect to food have been developed across the years. As happens with most of the general models, traditionally, the food models take a cognitive approach to consumer behaviour, where the decision-making process and the information processing of marketing stimuli are central to explain consumer behaviour (Verbeke, 2000). Marshall (1995) argues that while there is recognition of external influences such as product availability and economic factors, most food choice models focus on the interaction between the individual and the food product. The decision process (with a number of stages that varies with the specific model) is facilitated by information processing mechanisms and conditioned by psychological, social, cultural, and social influences that, usually, are afforded a peripheral role.

Holbrook and Hirschman (1982) state that the information processing models regard the consumer as a logical thinker who solves problems to make purchasing decisions. Various environmental and consumer inputs (e.g., products, resources) are processed by an intervening response system (cognition-affect-behaviour) that generates output consequences which, when appraised against criteria, result in a learning feedback loop. Individual differences, search activity, level of involvement and task definition affect the criteria by which output consequences are evaluated.

One of the most pervasive models concerning consumer behaviour towards food is the model proposed by Steenkamp (1997). His model distinguishes between the consumers' decision-making process with respect to foods, and the factors influencing this decision process. In the decision process, four stages are identified: need recognition, search for information, evaluation of alternatives, and choice. Three groups of factors influencing the decision process are recognized: properties of the food, factors related to the consumer, and environmental factors. For Conner *et al.* (1998) individual differences include psychological and physiological characteristics, individual resources, motivation and involvement, knowledge, attitudes, values and lifestyles. For meat, environmental influences integrate a wide variety of external stimuli (product information, price, branding, advertising, price, guarantees and labels, and country and region-of-origin) and the social and cultural context in which dietary choices take place.

More recently, Verbeke (2000) upgraded Steenkamp model to explain consumer fresh meat choices. In addition to the Steenkamp model this model is linked first with a "hierarchy of effects" model and then concepts related to information-processing are implemented. The "hierarchy of effects" indicates the different mental stages that consumers go through when making buying decisions and when responding to marketing or non-commercial messages. Verbeke argues that while it is generally agreed that a structure including a cognitive (learning, knowing), affective (thinking, feeling), and conative (intending, doing) component holds, no clear-cut evidence about the sequence and inter-dependency of these hierarchical steps appears to be available.

According to Hansen (2002), several researchers have suggested that the 'traditional', cognitive view should be complemented by taking into account consumers' affections, such as the possible emotional responses to the perception and judgement of products and of consumption experiences. Zajonc and Markus (1982) suggested that an individual can take action based on an emotional feeling that is without or with just a low level of cognitive activity. The reason for this is that positive emotions seem to affect consumer purchase behavior positively.

Garber *et al.* (2003) also recognize the complex nature of consumer responses to food products. However, they argue that there is an accompanying cognitive component to any sensory experience, in that prior experience with the same or similar products lends symbolic, associative and rhetorical meaning to any sensory experience. To support the cognitive information-processing perspective on consumer decision-making, it may be added that cognitions, according to Conner *et al.* (1998), might be beliefs about a food (e.g. about its health properties), attitudes toward a food (e.g. an overall evaluation), preferences for a food (e.g. plans to purchase or consume). Moreover, attitudes can have an affective component and are not, necessarily, formed on completely rational grounds (Marreiros, 2005).

In conclusion it can be argued that, in general, food choice and consumption are based on a cognitive decision-making process and take account of stimuli surrounding that choice and consumption. Past experience, sensory perception, and emotion or affect are important influences, in particular for food products but, at some point of the experience with the product, an evaluation based on some criteria (objective or not) is made by the consumers of that product. Depending on the product and on the situation the complexity of the choice may vary but, usually, there is a problem-solving approach to choice, even if affect or less rational factors influence the way people solve that problem (Marreiros, 2005).

The consumer decision-making process

According to Dewey (1910), the consumer decision-making starts with a felt state of deprivation or need identification. Than, the difficulty is located and defined and possible solutions are suggested and consequences are considered. Finally, a solution is accepted. As so, a typical decision-making process goes through different stages, from the problem or need recognition stage, to the outcomes of behaviour or post-purchase evaluation, passing through the information search or pre-purchase stage, the evaluation of alternatives stage, and the choice or purchase decision stage (Blythe, 1997; Schiffman and Kanuk, 1999; Solomon *et al.*, 2002).

Depending on the type of decision, the characteristics, knowledge and perception of the meat product, how information is obtained, the level of involvement with the product, and the consumer choice criteria, the process may be not an exact representation of the decision-making just described. In fact, sometimes, consumers simply do not go through all the typical stages of the process, either because they have very little time to buy meat products or because, in their view, the choice does not need a particular effort to be made. Thus, in order to reduce the level of effort necessary to make a choice, consumers choose a satisfactory option, rather than the optimal option (Solomon *et al.*, 2002).

According to Schiffman and Kanuk (1999), how information is obtained and is processed, i.e. the perception process is an important determinant of consumer decision-making. The information acquisition process entails the exposure of the consumer to stimuli produced in the surrounding environment. Depending on the importance of the issue involved, the individual may engage in an active search for information or be a passive receiver.

Every person, every day is exposed to an almost unlimited number of environmental stimuli. However, the individual sensory system produces sensation only for a specific range of stimuli. Additionally, the individual selectively devotes attention to a small proportion of the resulting sensations. Then, the sensations or raw inputs undergo an individual perceptual encoding process. This process develops personal meaning of the stimuli (information) that influences consumer decision-making. Loudon and Bitta (1993) identified five ways in which consumers use the information obtained: 1) understanding and evaluating products; 2) attempting to justify previous choices; 3) resolving the conflict between purchasing and post-purchasing evaluation; 4) satisfying a need for being informed about products; and 5) serving as a reminder to purchase products that must regularly be restocked.

The Problem Solving Continuum

One useful form of describing the decision-making process is to regard the magnitude of effort that goes into the decision and considering it as a continuum. This process starts with habitual or routine decision-making and finishes with extended problem solving, including, in the middle range, limited problem-solving (Solomon *et al*., 2002). The same three levels of problem solving (extensive problem solving, limited problem solving and routine response behaviour) are identified by Schiffman and Kanuk (1999).

The traditional decision-making process generally involves extended problem-solving, which is initiated by a motive fairly central to the self-concept of the consumer and in which, the eventual decision is perceived as carrying a certain degree of risk. If the consumers' knowledge about the products is very limited or non-existent and they do not have explicit preferences they try to collect as much information as possible, and devote time to reach a purchase decision. Given the importance of the decision, each product alternative is carefully evaluated in terms of salient attributes in order to construct a preferred option. Limited problem-solving is generally less complex. Consumers use simple decision criteria to choose amongst alternatives and are not motivated to search for information or to evaluate each alternative in detail (Schiffman and Kanuk, 1999; Solomon *et al*., 2002).

The above mentioned authors state that habitual decision-making takes place when consumers' knowledge and opinions about products are only partly recognized, and they are not completely able to assess product differentiation and establish a preference. Thus, a very low level of involved information search takes place and, also, little time is devoted to the purchase act and little or no conscious effort occurs. Some comparative information is required, while the decision criteria are, probably, moderately well defined. Some routine decision-making is made automatically, which means that consumers' knowledge and ideas about the product and its alternatives are well established. In this type of decisions there is a higher predisposition to purchase a particular product or brand and consumer decisions are performed with minimal effort and without conscious control (Schiffman and Kanuk, 1999; Solomon *et al.*, 2002). Finally, in numerous situations, consumer's decision is a learned response to environmental signs, cues, reminders or brands. That is the case of impulsive purchases, resulting for example from promotions or meat product offers, which can be seen as the extreme of the continuum.

Problem recognition

Problem recognition or need activation is the first stage of consumer-decision making. Much of consumer purchases and other behaviours are directed towards solving various sorts of problems. The consumer decision starts when the consumer recognizes a consumption problem that needs to be solved (Hyer and Macinis, 2001). Generally, problem recognition is understood as an awareness of the need to change the existing state to conform to the desired or ideal state. The individual's desire to solve a recognised problem depends on the magnitude of the discrepancy between the desired and the actual states.

Consumer problems can be active or inactive. An active problem is one the consumer is or will be aware of in the normal course of events, while an inactive problem is one of which the consumer is not aware. According to Engel *et al* (1995), a problem must be first activated before being recognized. A host of factors will influence the likelihood that a particular need will be activated. Such factors, as marketing influences, time changed circumstances, product acquisition or consumption or individual differences will operate by changing the individual's real and/or desired state.

2.3. Information search

Information search is the second stage in consumer decision-making. Once a problem which is likely to lead towards some action is recognised, the consumer engages in search for information that may assist him in decision-making. While information is the knowledge about some statement or fact, search refers to physical or mental seeking and processing information (Loudon and Bitta, 1993). Information search can also be defined as the encouraged activation of knowledge stored in the memory (internal search) or as the acquisition of information from the environment (external search) (Engel *et al,* 1995). The search for information may occur pre-purchase, post-purchase or be on-going.

Loudon and Bitta, (1993), state that information search, especially external search, varies among individuals and purchase situations. It also depends on the capability of the consumer to understand and evaluate the available information. Consumers will continue to search for information as long as they perceive the benefit of the search to be greater than its cost. The cost of information search includes, among other factors, time, foregoing other pleasant activities and money. The benefits of information search are that it gives consumers an assured feeling of making a decision, increases the chances of making a purchase that would give satisfaction, gives satisfaction of being knowledgeable about the product, gives pleasure in purchasing or consumption and can result in financial gains to be derived out of a purchase.

Factors affecting, directly and indirectly, information search are: market conditions (prices, style and appearance, alternative products availability and risk perception), consumer buying strategies (brand preference and loyalty), individual factors (confidence, experience, economic situation, capability to process information and beliefs) and situational factors (special offers, urgency of the product, store conditions, perceived risks and safety of the purchase) (Solomon *et al.,* 2002).

According to Steenkamp (1997), for food the most important information source is consumers' previous experience, i.e., internal search. External search for information is generally rather limited for making food purchase-decisions. The author argues that research has shown that the extent of information search is inversely related to prior purchase experience, involvement with the product category, time pressure, quality variation between product alternatives, and stability of the product category. All these factors tend to inhibit extensive search for information in the context of food products. Prior purchase experience is typically high, involvement relatively low, time pressure high, quality variation rather low, and there are comparatively few major product innovations or price changes. Engel *et al.* (1995) add that many food-buying decisions are actually made at the point of purchase. Consequently, in what concerns external search, in-store information can have a strong influence on consumer decision-making. Hoyer and Brown (1990) agreed that when involvement is low, information search prior to shopping, if undertaken at all, is often confined to such strategies as scanning. Therefore, it is critically important to firms to build brand awareness for the reason that people are significantly less likely to consider or to try an unfamiliar brand.

Alternative evaluation

The processes involving information search and evaluation allow the consumer to define alternative solutions and consequently come to a decision-making (Loudon and Bitta, 1993). Cant *et al.* (2002) state that consumer evaluation of alternatives is the act of identifying alternative solutions to a problem and assessing the relative merits and demerits of each, according to pre-established criteria and limits, which consumers decide are acceptable when searching for a problem-solution.

According to Engel *et al.* (1995) evaluative criteria are the standards and specifications which the consumers use to compare and evaluate different products and brands, which are important for them. In other words, evaluative criteria are the desired outcomes from purchase and consumption, and are expressed in the form of preferred attributes. These authors argue that an important aspect of understanding consumer decision-making involves identifying the particular evaluative criteria consumers use when deciding among purchase alternatives and to assess the relative salience of these criteria

Solomon *et al.* (2002) add that evaluative criteria are the dimensions used to judge the merits of competing alternative options, which are shaped and influenced by individual differences and environmental influences. Schiffman and Kanuk (1999) identify personality and attitudes as the most important individual influences to alternative evaluation, on the basis of which, four types of consumers may be defined. The first is the economic consumer, who makes a rational decision in light of complete information. The second type is the passive consumer, who is not knowledgeable and may be influenced by the marketer. The third type is the emotional consumer, who makes decisions entirely based on personal and irrational needs. Finally, the fourth type is the cognitive consumer, who makes decisions based on information from the environment, on social influences, on personal needs, attitudes, perceptions and previous experiences.

During the alternative evaluation stage, consumers first, decide which evaluative criteria to use, second, choose the alternatives to consider, third, calculate the performance of each alternative, and fourth, choose and validate a decision rule to find the final choice (Engel *et al.,* 1995). Satisfaction with the final choice may affect future evaluative criteria and future product evaluation. For these authors, motivation, involvement, and knowledge are individual factors that can determine the type of evaluative criteria likely to be used during alternative evaluation. Additionally, similarity of choice alternatives has an impact on the evaluative criteria used and on their relative importance for choice.

According to Loudon and Bitta (1993), evaluative criteria can be categorised as salient, determinant and critical. The salient criteria are those that are considered important by consumers. The determinant criteria are those that are considered very important. The critical criteria are those regarded as the most determinant attributes. Evaluative criteria may vary among categories of products and for food products Steenkamp (1996), reporting on a study involving 100 products and 7 European countries, found that that the five most important criteria used are product quality, price, brand name and reputation, freshness, and guarantee.

Loudon and Bitta (1993) and Solomon *et al.* (2002) state that the full set of alternatives actively considered during consumer's alternative evaluation can be separated into three subsets, namely, the evoked, the inert and the inept sets. The evoked set contains the few selected brands or products already in memory and the relevant options in the retail environment, which are positively valued by the consumer for purchase and consumption. The inert set is composed of those alternatives not under consideration, the ones where the consumer perceives no benefits and which are evaluated as neither positive nor negative. The inept set refers to alternatives of which the consumer is aware of, but that he would not consider to purchase, or to alternatives which have been rejected because of adverse reports or negative past experience.

The composition of the consideration set is a function of both personal and situational factors and of the interaction between them. Consumers will give greater consideration to brands that they perceive to be personally relevant, and which offer satisfactory performance on key attributes. Consumers are more likely to consider a brand that they can readily recognize or recall as a result of a recent, favourable, consumption experience or of direct or incidental exposure to advertising for that brand (Engel *et al,* 1995).

Moreover, the consideration set may vary with time, as consumers gain more information and experience. The search for information done by the consumer and the display of information through marketing communications made by the marketers, increase the range of alternatives known to the consumer. Loudon and Bitta (1993) explain that certain alternatives may not capture consumers' interest because: they may be beyond consumers financial means; may not be suited to fulfil consumers needs; consumers may lack information for a complete alternative evaluation; consumers might have been unsatisfied in the past with the product; consumers might have received a negative feedback about the alternative; or consumers might perceive their current brand or product as good option.

When the consumer makes a product or brand choice among a set of alternatives, a number of decision rules may be used. As general rule, Loudon and Bitta (1993), argue that the greater the urgency of the need, the lesser is the evaluation process; the greater the significance of the product, the lesser is the evaluation process, the greater the complexity of the alternatives, the greater is the evaluation process. According to Solomon *et al.* (2002), when using non-compensatory rules, consumers eliminate alternatives that do not comply with any of the criteria the consumer has chosen to use. In other words, the weaknesses of the alternatives in salient criteria are not compensated by possible strengths in other attributes. On the other hand, compensatory rules, which are more probably applied in high-involvement situations, allow the decision-maker to consider each alternative product or brand as an all, and consider simultaneously the all set of salient attributes to arrive at the best overall choice. Cant *et al.* (2002) conclude that evaluation brings the consumer to the point of making decision concerning a specific course of action.

Relating to meat, Furst *et al.* (1996) identified six values (criteria) that could be determinant in consumers' evaluation: sensory perceptions, monetary considerations, convenience, health/nutrition, managing relationships, and quality. In accordance with the results of most food research, these authors found out that sensory perceptions were often the dominant value and were driven mostly by taste. Sensory perceptions often served as the limiting factor in food choice, and tended to be less negotiable than other values. Sensory perceptions, particularly taste, and monetary considerations were frequently in conflict. Taste was also often weighed against convenience.

Influencing consumers' alternative evaluation is not easy. Consumers' well-established evaluative criteria are difficult to change and criteria that are contrary to consumers' common sense will not generally accepted by a large market segment. From a marketing perspective, an important task of the marketer is to influence consumers' evaluative process, gaining good evaluations for the brand and with that increasing the chance of being consumers' final choice. For that, first and foremost is the job of the marketer to locate the product or brand in the evoked set of alternatives, through marketing communication and the creation of good consumption experiences (Schiffman and Kanuk, 1999).

Purchase decision

The result of the evaluation process is a preference for a product. Preference implies choice and decision; to prefer a product is to choose it over another (Rozin, 1990). Decision is to select from two or more alternatives and act, which means to take the most preferable and feasible decision. For Kardes (2002), a decision involves selecting one product from a set of possibilities. Consumers either purchase the product or they do not, there are no in between. Of the alternatives which have been generated and evaluated, a choice and a decision must be made.

Cant *et al.* (2002), state that consumer decision is the outcome of evaluation and involves the mental process of selecting the most desirable alternative from a set of options that the consumer has generated. Furthermore, these authors argue, the most suitable choice is the one that comes closer to the evaluative criteria defined by the consumer. According to Rozin (1990), in more prosperous cultures, as availability and cost recede in importance, preference is more in line with use. For Steenkamp (1996), food choice is in accordance with attitude theory, which means that, for each consumer, the product alternative holding the most positive attitude will be the chosen product. However, there are a number of factors that weaken the relation between attitude and choice in the context of foods. Pressures from the social environment, the degree of behavioural control, habits, and variety-seeking behaviour are some of these factors.

Many consumers have consistent choice patterns. Some, due to inertia, tend to purchase the same product or brand almost every time. In contrast, others are loyal to brands and repeat purchasing behaviour as a result of a conscious decision to continue buying the same brand. Compared to inertia, where the consumer passively accepts a brand, a brand-loyal consumer is actively involved with his favourite brand (Solomon *et al.*, 2002). However, brand parity, one of the main causes of inertia, is one of biggest challenges that marketers are facing nowadays. Brand parity refers to consumer's beliefs that there are no significant differences between brands.

To understand consumer decision to purchase or refuse a product is essential for marketers. It is practically impossible to persuade a consumer to purchase anything unless he can see some benefit to himself flowing from that action. When consumer purchase products the physical item itself is irrelevant - instead he is buying the benefits that product will give. Understanding which benefits are central for the consumer and which attributes of the product are preferred, is vital in marketing management and constitutes the foundation for the of marketer's strategy success Schiffman and Kanuk, 1999).

Pos-purchase evaluation

Pos-purchase is the final stage in the consumer decision-making process. To the consumer, a purchase results in expenditure and in getting products; products which the consumer expects will fulfil needs and solve the problems recognised in a previous stage. The results of the purchase may have more than one dimension and post-purchase activities can include consumption, evaluation and divestment (the disposal of unconsumed products or their remains).

Cant *et al.* (2002) state that pos-purchase behaviour entails consumer evaluation of the product performance once it has been bought. Post-purchase evaluation results in consumers' perceptions of the outcome of the consumption process. Schiffman and Kanuk (1999) grouped pos-purchase evaluation into three groups. In the first group, the performance of the product is as expected and the feeling of the consumer will be neutral. In the second group, the product performance may is above expectations causing a positive disconfirmation, which leads to satisfaction. In the third group, the product performance is below expectations causing a negative disconfirmation, which leads to dissatisfaction.

The consumer expects satisfaction of a purchase. Loudon and Bitta (1993), stress that satisfaction is strongly dependent of expectations. One could have a pleasant experience that caused dissatisfaction because even though pleasant, it was not as pleasant as expectable. According Cant *et al.* (2002), satisfaction occurs when the outcome and the conditions surrounding the product are matched with consumer's expectations. In the same context, consumers express dissatisfaction when the outcome does not match their expectations or when they feel that the product fells short of expectations in important aspects. In their research, Raats et al. (1995) concluded that satisfaction with the product is influenced by the effort (which includes the physical, mental, and financial resources) expended to obtain the product, and the expectations concerning it.

Cant *et al.* (2002) further explain that post-purchase involves different forms of psychological processes that the consumers experience when purchasing a product. According to them, post-purchase learning is one of such processes. After purchasing a product, the consumer discovers something about it or about the store where they bought it, and this new knowledge may be stored in consumer's long term memory, modifying relevant attitudes and staying available to use in the next decision process as an improved base of knowledge. Consequently, product dissatisfaction or satisfaction may influence consumer future decision-making process. Loudon and Bitta (1993), state that product satisfaction will probably result on further positive pos-purchase behaviour, on increased purchase intentions and on increased brand or product loyalty. However, if the consumer is dissatisfied with the product or brand, the post-purchase attitude will be negative. Moreover, some consumers may exhibit complaining behaviour and spread negative word-of-mouth.

Given that food is a category of products where the choice and consumption can be included in habitual decision-making process, the result of past experience is determinant for future choices. As Grunert (2003) argues, since many food items are frequently-bought products, previous sensory experiences affect future purchases, but then it is the beliefs formed due to previous sensory experiences which affect the purchase, not the experience itself. Cardello (1995) found as well that when the taste experience with a product fails to match the expectation of the product, the result is a disconfirmation of a subject's expectation. Raats et al. (1995) concluded that all food products, the pattern of disconfirmation or confirmation of the expected sensory properties is likely to prove a critical factor in the decision to repurchase.

Consumers may also experience post-purchase dissonance. Loudon and Bitta (1993) define post-purchase cognitive dissonance as a psychological state of mind which occurs as a result of discrepancy between the consumer's decision and its prior evaluation. Cognitive dissonance creates an individual state of tension and the individual may respond harmonising the conflict and, consequently, reducing tension. Cant *et al.* (2002) state that some consumers, who make poorly reflected choices, may experience post-buying conflict, questioning their decision and product purchase. Others, particularly after making a significant, complex and quite permanent decision, may experience doubt or anxiety, which is defined as post-buying dissonance. According to Hawkins *et al.* (2002), consumers try to reduce post-buying dissonance changing cognition and attitudes. By looking for helpful information or distorting information concerning a specific product, consumers will find their psychological equilibrium.

The post-purchase evaluation stage also includes the disposal of products after consumption or use. Actually, the disposal of products also goes through a process of problem recognition, information search, alternative evaluation, disposition decision and pos-disposition results (Loudon and Bitta, 1993). Consumers may choose a specific product because of the way they wish to dispose of it, trade it off or keep it temporarily or permanently. Very often marketers provide information and support considering disposal of products. Disposal of products is also a concern, in terms of environmental policies or convenience to citizens, for policy makers and government authorities.

FINAL COMMENTS

In general, choice and consumption of meat products is based on a cognitive decision-making process and take account of environmental stimuli surrounding that choice and consumption. Past experience, sensory perception and emotion or affect, are important influences, but, at some point of the experience with the product, an evaluation based on some criteria (objective or not) is made by the consumers. Depending on the meat product and on the situation, the complexity of the choice may vary but, usually, there is a problem-solving approach to choice, even if affect or less rational factors influence the way people solve that problem.

Attention should also be paid to potential influences to consumer decision-making that might result from communication and marketing stimulus and previous meat choices, which provide information and experience that becomes input for future choices. Thus, factors influencing the decision process can be properties of the food, factors related to the consumer, environmental factors (Seenkamp, 1997), and information-processing related to marketing stimulus (Verbeke, 2000). Therefore, meat choice results from a meat evaluation process based on its attributes, which is informed by attitudes, beliefs and motivations and other environmental or individual factors. For Furst *et al.* (1996), food decision-making can be highly reflective or habitual and automatic, with a bundle of influencing factors to choice, including the social-context, monetary resources and people's life-courses experiences.

Safety and health related issues also influence consumers' attitudes and behaviour relating to meat products (Lucas, 2006). Consequently, meat safety and freshness are determinant in defining meat experienced quality. In an empirical research with beef consumers, Marreiros (2005) found that freshness and butcher's advice are very important cues for meat buying-decision. Colour is the other main cue for choosing meat. The importance of fat as an attribute for experienced meat quality and as a choice cue is not confirmed in the study, and consumers' awareness and knowledge about the quality labels and about the PDO meat brands was found to be rather low. On the other hand Lucas (2006) concluded that these guarantees or certifying labels are valuable decision-making criteria for some Mediterranean consumers segments (Lucas, 2006). The country-of-origin or region-of-origin of meat are product attributes with complex effects on consumer behaviour. They have high symbolic meaning, which reflect geographical differences in the food culture, both between and within countries.

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DYNAMICS AND SOCIO-ECONOMICAL VALORISATION OF THE LOCAL CELTIC PIG BREEDS.

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SUMMARY - In the last century the European pig production systems underwent intensification in order to satisfy massive demands for lean meat. In parallel, Europeans local pig breeds, including Celtic pig breeds, declined due to their lower production ability and poorer adaptation to intensive farming. Celtic pig breeds are spread, basically, over the European Atlantic Arc regions. Generally these ancient genetic resources have poor growth rate and carcass conformation and, even though the quality and suitability for processing of their meat is widely acknowledged, most of them became extinct during the second half of the 20th century. At present, breeders, consumers and market operators are increasingly considering that new traits, such as intramuscular fat content, nutritional value or fat quality, are interesting to allow diversification of products in pig industry and improvement of marketing possibilities. Moreover, the European consumer demands high quality food products obtained using production systems compatible with the preservation of environment, landscape and genetic resources. In this respect, traditional food products obtained using local livestock breeds and traditional practices can contribute to achieve these goals, and they can also have significant effects on innovation and added value for marketing and rural societies. In this paper we tried to point out the social dynamics and the population needs of the Western Europe Celtic pig breeds: Portugal (*Bísaro* and *Malhado Alcobaça*); Spain (*Celta*, *Asturcelta* and *Euskal Txerria* in Galicia, Asturias and Basque Country; respectively) and France (*Blanc de l'Ouest*).

Key words: Socio-Economical, Portugal, Spain, France, local Celtic pig breeds.

INTRODUCTION

In the last century the European pig production systems underwent intensification in order to satisfy massive demands of lean meat. Pig production systems use now standard facilities, feeding methods, housing techniques and genetics. In parallel, European local pig breeds, including Celtic pig breeds, declined due to their lower production ability and poorer adaptation to intensive farming.

Celtic pig breeds are spread, basically, all over the European Atlantic Arc regions. In general, these ancient genetic resources have poor growth rate and carcass conformation scores and, even though the quality and suitability for processing of their meat is widely acknowledged, most of them became extinct during the second half of the 20th century due to crossbreeding between local Celtic pigs and improved breeds with high muscular development, feed efficiency and percentage of lean meat. Although production ability has increased, these crossbreedings have caused a major decrease on sensorial and technological quality of the pork meat. At the present, breeders, consumers and market operators are increasingly considering that new traits, such as intramuscular fat content, nutritional value or fat quality, are interesting to allow diversification of products in pig industry and improvement of marketing possibilities. Moreover, European consumer demands high quality food products obtained using production systems compatible with the preservation of environment, landscape and genetic resources. In this sense traditional food products obtained using local livestock breeds and recovered traditional practices can contribute to achieve these goals, and they can also 1have significant effects on innovation and added value for marketing and rural societies. In this paper, we point out the present situation and needs of the local Celtic pig breeds in some regions of the Western Europe.

What is the celtic pig breed type?

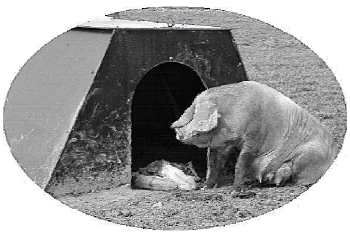
There are many issues suggesting the existence of a Celtic group within the local European pig breeds. They probably share a common genetic origin (Royo et al., 2007) but also basic morphological and phenotypical characteristics. Moreover, they are spread in areas with strong geographical links, similar climate, rural context, livestock production systems, and cultural references.

The name "Celtic" was proposed and applied by Sanson to express the antiquity of this unique type that existed in all Celtic countries (Gallia, Britain Islands, Northern Iberia) before the introduction of other types such as Romanic and Asiatic breeds (Bernardo lima, 1856). For centuries the autochthonous Celtic pig breeds have spread all over Northern Portugal, Spain and the Atlantic countries in Western Europe. This pig strain has become nearly extinct in the second half of the last century because they are fatter, less productive and poorly adapted to intensive farming than improved pigs. Understandably, Celtic pigs can be classified into the European unimproved domestic pigs that were in the origin of all modern breeds and hybrid commercial swine lines.

Morphology

Swine's belonging to the *Celtic group* are braquiocephalic animals, with long body, convex back and flat sides. Celtic pigs have a well developed skeleton with musculature more developed in the front part of the body, with strong shoulders and a big head. Particularly, ears are very long and floppy covering the eyes. Often animals have abundant and thick hair with a great variety of skin colour. All remaining local Celtic pig breeds (*Bísaro, Malhado Alcobaça, Celta, Asturcelta, Blanc de L'Ouest*, etc.) include well-sized individuals with large body and long legs, big and flopping ears covering the eyes and convex back. The Celtic pig population has several varieties of skin colour; they can be blue-grey, white or spotted (see set of Fig. 1 and Fig. 2)).

Figure 1. Local Celtic pig breeds within Portugal and Spain.



Bísaro (Portugal)

Malhado de Alcobaça. Portugal

Asturcelta or Gochu'l País – Asturias (Spain)

Figure 2. Local Celtic pig breeds within Spain and France.

Celta in Galicia
(Spain)

Euskal Txerria – Pays Vasco
(Spain)

Blanc de L'Ouest. (France).
Photo crédit IFIP

Brief description of each breed

- ***Bísaro* –** Similarly to the morph type of the ancient Celtic pigs, the Bísaro breed has a large body and long legs; they are big, sometimes flabby, with big and flopping ears, a concave head and a convex back. This breed has several varieties of skin colour; they can be Blue-Grey, white or spotted, with or without belt. This local Portuguese breed has poor growth rate and bad conformation, but it is traditionally known for its excellent meat quality, suitable for processing.

- **Malhado de Alcobaça** – This breed has a large and concave head with flopping ears covering the eyes. The animals have a well developed skeleton with a strong and long body with a convex back and a strait rump. Concerning skin colour, these animals have black spots of medium and irregular size spread over the whole body, except generally the extremities of the body. Carcass is leaner than the other Portuguese Celtic breed (Bísaro).

- **Celta (Galizia) –** Animals of this breed have a straight profile, large ears, a convex back and large limbs. They are white and white with black, sometimes spotted. This breed is well adapted to mountainous regions and is known for its rusticity and its prolificacy and for its meat quality (DAD-IS www.dad.fao.org).

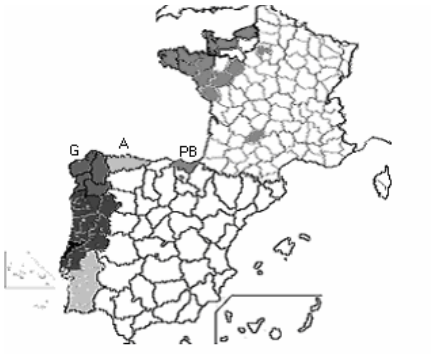
- **Asturcelta (Asturias) –** Animals of this Celtic pig breed are big, wide and long-headed with sub-concave profile, small eyes, and long ears falling to the front and covering the eyes. Long body with convex back and flat ribs. Long and bony legs and a long and strait tale. White, black or spotted coat (Sevilla, 2001).

- **Blanc de l'Ouest** – **(France)** - The Blanc de l'Ouest pig breed has its origin in the end of the 50's by genetic fusion of several French local pig breeds (Craonnaise, Normande, Flamande and few other varieties). Of Celtic origin, it has a white coat with a tuft on the back, a concave profile, ears flopping forward over its eyes, and a deep chest. Individuals are big (boar 450 Kg; sow 350 Kg), well adapted to outdoor conditions and they are known for their good meat quality and aptitude for cured and cooked meats.

- **Euskal Txerria –** This Celtic pig breed is close to the Chato Vitoriano and Chato Baztanés pig breeds that are, at present, extinct. The breed has spotted coat with average height at withers of 0.75 m, height at pins of 0.78 and body length of 1.40 m. Long headed, straight profile and narrow front. Large and flopping ears, covering the eyes.

Geographical distribution of the Celtic pigs (Portugal, Spain and France)

Figure 3. Geographical distribution of the six local Celtic pigs breed in Europe:



i. Portugal – Malhado de Alcobaça breed is in black and Bísaro is in grey;
ii. Spain - Regions of Galicia (G) (Celta pig breed); Principado de Asturias (A) (Asturcelta breed); Pays Vasco (PB) (Euskal txerria);
iii. France – Blanc de l'Ouest in Normandie and Bretagne; Source IFIP, www.ifip.asso.fr.

The local Celtics pig breeds in each country/region - Portugal, Spain and France.

Table 1. Local pig Breeds belong to the Celtic pig type.

Country Region	Name of the Breed	Variety	Number of animal in Herdbook (year 2007)			Herdbook established (Year)	Status of the breed (FAO)
			Adult males	Adult females	Farmers		
PORTUGAL	Bísaro	Galega or Minhota	289	1736	99	1995	Endangered
		Beiroa					
		Trasmontana					
	Malhado Alcobaça		11	160	1	2004	Critical
SPAIN							
Galiza	Celta	Santiaguesa	150	600	210	2000	Endangered maintained
		Barcina Carballina					
Asturias	Asturcelta	Asturcelta or Gochu'l País	22	30	23	2005	Critically endangered
País Vasco	Euskal txerria	Basque, Bearnesa, Bigurdana	5	30	5	2007	Critical
FRANCE	Blanc de l'Ouest		35	110	30	1996	Endangered maintained

Extinct local Celtic pig breeds: Podolienne, Bohême, Dano-scandinave, Bavaroise, Bakonyer, Flamende, Bretonne, Craonaise, Augeronne, Normande, Irlandese, Lorraine.

Livestock production system

- **Bísaro –** A high range of housing systems – indoor, outdoor, intensive, extensive, and a great diversity of building structures are used by farmers. Nevertheless, the majority of farms where Bísaro breed can be found are the small traditional units in the interior north of Portugal or in mountain regions. In the last five years the number of units using the outdoor system (more or less intensively) was increasing, which allowed for an increase in the stocking rate per unit. A new work programme using Bísaro breed as a part of the organic pig production is in held.

- **Malhado de Alcobaça** – This breed is currently reduced to a single herd (a private company) with 170 sows in a classic intensive system – confined in individual stalls.

- **Celta (Galicia)** – This genotype can contribute to the valorisation of grassland and deciduous forest as in other countries. Animals can be reared in a free-range system or in an intensive or semi-intensive outdoor system. Some times this local breed is reared in industrial farms together with exotic breeds.

- **Asturcelta (Asturias) –** The breed is maintained in several small farms each of them including 1 boar and from 1 to 5 sows. Natural mating is the only reproductive system used. Fattening is carried out in semi-extensive conditions using natural products.

- **Euskal Txerria –** The Euskal Txerria breed originated in the eastern part of the Pyrenees extending to the territories of Iparralde and Hegoalde. Its revival occurred in the Valley of Aldudes, in the Lower Navarra. Nowadays it has a stable population in its area of origin and it is gradually increasing in the territories of Navarra, Gipuzkoa and Bizkaia. It was a usual practice to fatten some of the animals for family consumption using home grown maize as the main food source during the fattening period.

- **Blanc de L'Ouest –** The Blanc de l'Ouest pig breed is reared outdoors, in small farms sometimes converted to biological agriculture. Most often, farmers breed 2 or 3 sows and 1 boar. The association that regroups farmers helps them to buy and replace boars. Each farmer has got a boar, indeed artificial insemination is not in use in order to control the number of issues per boar and then to preserve genetic variability. The number of reproducers has not increased for many years because of lack of market outlet.

DYNAMIC AND STRATEGIES FOR BREEDS' CONSERVATION

Genotypes conservation as static and *in situs* methods are used for each breed and countries (tab. 2).

Table 2. Genotypes conservation dynamic and strategies used in each local breed/country.

	Static						Dynamic		
	Reproductive cells			Somatic cells	DNA data bank		In vivo Conservation		
	Semen	oocytes	embryos	Tissues	Somatic	Mitochondrial	Experimental Station	Farmers	park Zoo
Bísaro	n	n	n	y	y	y	y	y	y
Malhado de Alcobaça	n	n	n	y	y	y	n	y	n
Celta	n	n	n	n	n	n	y	y	y
Asturcelta	y	n	n	y	y	y	y	y	n
Euskal Txerria	n	n	n	n	y	y	y	y	y
Blanc de l'Ouest	y	n	n	n	y	n	n	y	y

- **Portugal** – In the 90's the Bísaro breed was almost extinct (around 200 individual) and the first Portuguese programme for its conservation and recovery was approved and support by the government in 1997 (Pires da Costa *et al.,* 2001). The enthusiastic results obtained with this project contributed to follow-ups of the conservation programme and development of the new farmers' initiatives, as to expand and improve traditional and outdoors livestock system. Today with more than 200 boars and 1000 females, these breed is used and preserved *in situs* by farmers with assisted management of the breeders' association (Associação Nacional de Criadores de Suíno Bísaro - ANCSUB) and government (Ministry of Agriculture) contributing to the economical income of the rural societies and families. For now, an increase of the typical pig meat chain based on Bísaro breed is expected.

- **Galicia** – The interest of the recovery of the Celta breed is backed by the autonomous Regional Government of Galicia, who-conscious of the genetic, social, cultural, and economic loss that the disappearance of this breed would mean- has directly or indirectly coordinated and put into action the infrastructural and technical measures necessary to the recuperation of this breed. Their course of action also brought about a latent implication of farmers in the recuperation program which was confirmed in early 1999 with the creation of the Celta livestock breeders association (Asociación de Criadores de Ganado Porcino Celta, ASOPORCEL) by the city council of Triacastela, in the province of Lugo (Carril *et al.,* 2001).

- **Asturias** – In the end of the 90's only 20 Asturcelta pig individuals could be identified in traditional farms of Asturias. Since 2005 and based on these animals (2 boars and 6 sows) a Multiplication Nucleus of the Asturcelta pig breed was built and managed by the Government of Principado de Asturias through SERIDA in Villaviciosa (Agri-Food Research Institute of Asturias) in close collaboration with the breeders association (ACGA born in 2002). At the present, various conservation lines including semen extraction are held in this nucleus.

- **Basque Country –** In Iparralde there is a breed association - *Association des Eleveurs de Porcs Basques* made up of farmers from the Aldudes valley, and there is another in Bidania (Gipuzkoa). Within the project for the recuperation and conservation of the breed, a conservation farm was created, located in Aldudes, with capacity for 30 sows and 30 boars. From the offspring obtained, one female and at least one male of each family are kept on the farm, either alive or as frozen gametes. Whenever a farmer needs to replace a boar, the conservation farm provides him with one (Gómez, 2003).

- **France –** The national preservation program of French local pig breeds initiated jointly by IFIP (ex ITP) and INRA in 1982 allowed the census of Blanc de l'Ouest pig breed: 169 females and 10 boars in 84 farms. In 1994, farmers created an association which helps to preserve this breed. Following, LIGERAL (pig local breeds herd book, constituted in 1996) collected and compiled pedigree information in an informatic data base at IFIP. Now, 110 Blanc de l'Ouest sows and 35 Blanc de l'Ouest boars are reared in 30 farms. Animal numbers of are still small because of the difficulties to find outlet. In 2006, frozen semen from 2 boars born in the end of the 80's was thawed in order to reconstitute extinct male lines. Four young boars are currently in use in farms and two others are in an artificial insemination centre to freeze their semen.

MEAT PRODUCTION AND MARKET

Traditional pig meat production has been an economically important activity in rural areas of the Celtic regions. In this respect, traditional technical knowledge is still present in rural populations of the areas in which Celtic pig breeds are spread. Recovery of the Celtic pig breeds should be parallel to the recovery of these traditional production techniques all together with the addition of environmental production techniques and market goals.

Generally local Celtic pig breeds have poor growth rate and carcass conformation, high (no-commercial) adult weight and body size. However, they can have a market niche within the pig industry because of their well known meat quality and suitability for processing traditional products in a market scenario in which consumers demand safe products with differential quality obtained from methods that contribute to the environmental preservation.

A putative label "*Celtic Pig Meat Products*" would include a great number of different products (fresh and processed) all of them unique in the world. Consistency of the label would be based on the fact that typical Celtic pig meat products share similar features and origin in terms of genetic, geography, technology, history and cultural references.

Products

Since 1991/92 typical products in Europe are susceptible to be protection and labelling by specific legislation, as a Protected Designation of Origin, a Protected Geographical Indication (2081/92/CEE), and a Certificate of Specific Character product (Reg. CEE 2082/92) and also, labelling as organic production (Reg. CEE 2092/91). Till then, each typical product and chain under administration of its organizations had its own history and affirmation on market. Below, we present a list for protected and non-protected traditional pig products obtained from the Celtic pig breeds, for each country and region.

Portugal

Protected Products (15 products) – Chouriça de carne ou linguiça de Vinhais; Chouriço azedo de Vinhais; Chouriça doce de Vinhais; Butelo de Vinhais; Alheira de Vinhais; Salpicão de Vinhais; Alheira de Barroso; Chouriça de carne de Barroso; Chouriço de abóbora de Barroso; Salpicão de Barroso; Sangueira de Barroso; Alheira de Mirandela; Presunto de Barroso; Presunto de Vinhais; Carne de porco Bísaro transmontano (fresh meat). These products are sold in farmers and small family industries (23 traditional farmers).
Others products using traditional recipes and Bísaro breed (Celtic type) or their crossbreeding can be found in the market without protection or trademark.

Spain

Asturias - A Multiplication Nucleus of the Asturcelta pig breed is managed by ACGA and the Government of Principado de Asturias through SERIDA.

Galicia - Its association of livestock breeders (ASOPORCEL) is supported by the Regional Government of Galicia, and more significantly the quality of its meat is socially and scientifically recognized as demonstrated by the social interest it has triggered and the demand for pigs. Together these factors foretell a promising future for this well-known inhabitant of barns and fields in Galicia.

Basque Country/Euskal txerria - Embutidos: i. Morcillas/odolkiak: de arroz, de puerro y tripotak; Lope/morcillón/moscanzo/lopon/guitalope/Aite eterno/Jaungoiko/Jaungoiko nagusi/trinpoila; Culón o morcilla cular; Relleno; Mondongo; Buskentza/odoloste; Tripota/odolgi; Retuertos; *ii.* Chorizos/lukainkak: Txorizoa/txorizua/de domingo; Birika/biris/biriki/biriquis/birizko/birica/biriki-lukainka/sabadeños /de sábado; *iii.* Lukinka/lukainka; Txistorra; Salchichón; Buru-gasna; Jamón; Paté.

France

The aim of the breeders' syndicate that regroups 30 farmers is to save the Blanc de l'Ouest pig breed with no commercial objective. Each farmer sells his products on his own (mainly cooked products: pâté, rillettes): sale on farm, at the market. Further more, traditional restaurants propose menus with fresh pork (essentially suckling pig).

SOCIAL AND CULTURAL EVENTS

Concerning the researches on the local Celtic pig breeds many events have been developed in the last decade in Portugal, as well in Spain or France. In all these countries in the natural pig breed regions there are a lot of technical events as regional meetings, seminars, demonstration and training activities linked to the local breeds and typical products (Fig. 4 and 5).

Figure 4. Examples of the economical and social dynamic on the natural pig breed regions.

National contest of Bísaro breed (Vinhais; Portugal)

Blanc de L'Ouest. Au Salon International de l'Agriculture de Paris. Photo crédit CDMP.

Fair of typical products in Vinhais; Portugal

Figure 5. Examples of the economical and social dynamic on the natural pig breed regions.

Euskal txerria Patea
(Pays Vasco)

Fair of typical products
(Melgaço; Portugal)


Organoleptic test during a
meeting Portugal and Spain

Economical and social dynamics of this sub-sector can be seen in many trade fairs, flea markets, industrial exhibitions, show-rooms and even national and regional pig–shows, all this followed by cultural events. For example, in France: Salon International de l'Agriculture de Paris (Concours général agricole)*;* Salon des races locales à Ménez-Meur (concours régional) ; Fêtes de la vache nantaise au Dresny (concours régional); In Portugal: in the natural pig breed region there are a lot of technical events under the supervision of ANCSUB and Research Centres, as National Concourse du Porc Bísaro in Vinhais ; Market exhibitions in National fair of Montijo ; Annual fairs of typical products in Vinhais, Melgaço, Montalegre ; Boticas, Cabeceira Basto.

CELTIC PIG BREEDS ASSOCIATIONS

Portugal

- *Bísaro* – ANCSUB (Associação Nacional de Criadores de Suínos da Raça Bísara) Morada: Edifício da Casa do Povo, Largo do Toural; 5320-311, Vinhais – Portugal; Tel.: 273 771 340; Fax.: 273 770 048; e-mail: ancsub@mail.telepac.pt; web: www.porcobisaro.net;
- *Malhado Alcobaça* **–** Federação Portuguesa das Associações de Suinicultores / APCRPS web: www.suinicultura.com

Spain

- *Galiza (Celta breed)* – ASOPORCEL (Associación de Criadores de Ganado Porcino Celta) – Spain - Galiza; http://www.porcocelta.com/;
- *Asturias (AsturCelta)* - ACGA (Associación de Criadores Gochu Asturcelta**)** Spain – Principado de Astúrias http://www.serida.org/publicacionesdetalle.php?id=01503
- *País Vasco (Euskal txerria)* – ETE (Euskal Herriko Euskal Txerriaren Elkartea. Bº San Miguel 11-1º. 48200 Garai (Bizkaia) (Spain). Web: www.euskalabereak.com

France

- *Blanc de l'Ouest* - Syndicat des Éleveurs de Porc Blanc de l'Ouest - Parc Naturel Régional d'Armorique - 15 place aux Foires - BP 27 - 29590 LE FAOU - 02.98.81.08.88 ; (Secrétariat: M. Sergent, Président: M. Luquet).

CONCLUSIONS

The recovery of the local Celtic pig breeds should be parallel to the recovery of the traditional production techniques all together with the addition of friendly environmental techniques and market goals.

A putative label **"*Celtic Pig Meat Products*"** would include a great number of different products (fresh and processed) all of them **unique in the world**. Consistency of the label would be based on the fact that typical Celtic pig meat products share similar features and origin in terms of genetic, geography, history and cultural references.

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PURCHASING BEHAVIOUR OF FRENCH AND SPANISH CONSUMERS' TOWARDS DRY-CURED HAM

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SUMMARY - Nowadays, reaching consumers' expectations is a major issue for both strong brand products and local products linked to their region of origin. Although the results from many studies show high commitment stated from consumers towards local products, the main question which still exists is which attributes influence the actual purchase behaviour and the behavioural loyalty. We have used panel data, in the framework of the EU funded project TYPIC, to analyse those issues. Data were collected over one year in French and Spanish supermarkets, to measure actual purchase and loyalty on dry-cured ham. The results show that quality labels is not the most important attribute influencing behavioural loyalty. For most consumers, behavioural loyalty seems to be still under the influence of price impact. However, some of the analysed panellists are not price sensitive, but still have various preferences and variety seeking behaviour.

Key words: Consumers, Purchasing behaviour, Quality labels, Dry-cured ham

INTRODUCTION

Dry-cured ham is eaten along Europe but mainly in the Mediterranean countries. Due to its relatively high price, compared to other usual food products, dry-cured ham consumption is constrained to budget limitations. As a consequence it is sold in small packages. Thus, it is interesting to find out the reasons behind the familiarity of European consumers with dry-cured ham and their low purchase frequency. Spain has the world record of dry-cured ham consumption: 4.5 kg per capita per year. France is known to be one of the most important countries where consumers have high experience of quality labels highlighting the origin of food products.

This paper aims to cross-tabulate consumer behaviour responses of Spanish and French dry-cured hams. We will present successively the rationale of the study, the scientific protocol and the results from France and Spain focusing on consumer purchasing behaviour. Finally, a Bayesian analysis with respect to purchasing behaviour will be shown.

MATERIAL AND METHODS

A consumer survey was carried out over one year in French and Spanish supermarkets during 2004. Clients were recruited among the users of their own supermarkets credit cards. This allowed to record scanned purchase data. In both countries, the monitored hams were those available on the self service shelves of the supermarkets, and sales from the delicatessen shelves were also recorded in Spanish supermarkets. All panellists bought at least one package of dry-cured ham during the three months before the recruitment period. The observed categories of dry-cured ham were Serrano, Teruel DO, Teruel no DO, Iberian no DO, TSG Serrano and Iberian Paleta no DO in Spain. In France they were Bayonne PGI, Lacaune (DO candidate), Aveyron, Auvergne, and also strong brands with no region of origin. Brands were classified into distributor, strong commercial, local and no frills brands.

In Spain, the panel consisted of 5640 shoppers who bought dry cured ham, along one year, from two hypermarkets in Zaragoza. The entire set up included 91 different references. During the observation period the total number of packages bought was 19599 with a total turnover 166489 €.

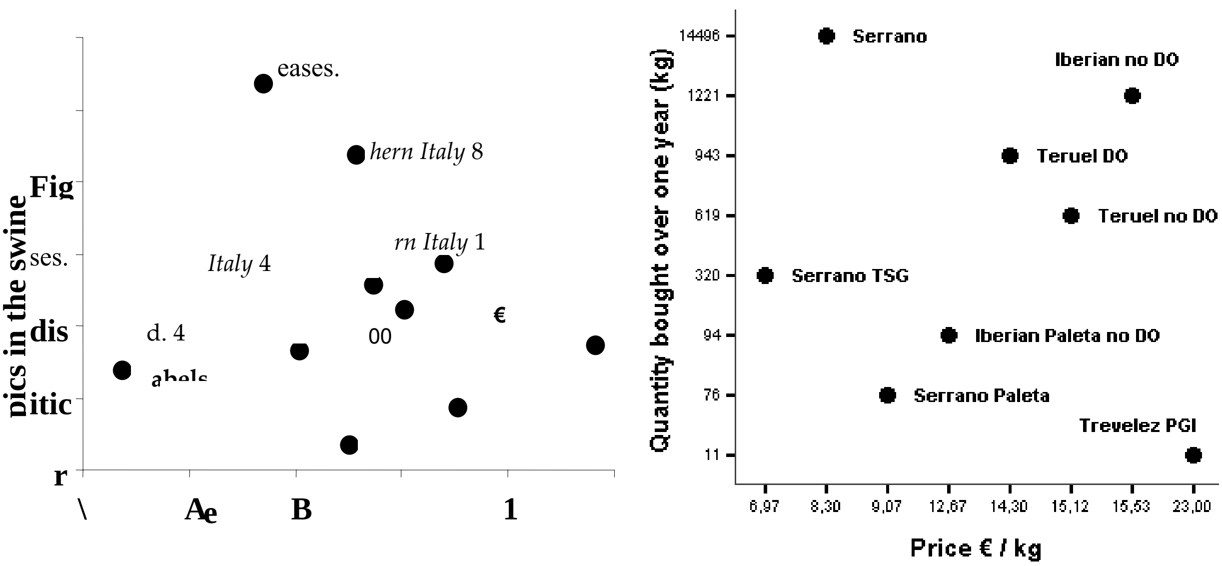
The average amount spent per panellist was approximately 29.52 € and the average packages or pieces bought was 3.5 per buyer. The prices ranged from 3.14 to 83.50 €/kg. In France, the panel consisted of 778 shoppers who bought dry cured ham, along one year, from four supermarkets in the Auvergne region. There was 10 different references. During the observation period the total number of packages bought was 4674 with a total turnover 16659 €. The average amount spent per panellist was approximately 21.41 € and the average packages bought was 6 per buyer. The prices ranged from 1.38 to 5.83 €/kg.

RESULTS AND DISCUSSION

Buying preferences

Results show that buyers are quite price sensitive and basic criteria to decide about the volume of their purchases were price and brands. As usual, the demand curve is price sensitive although there is a noticeable segmentation of the ham displayed in the stores' shelves. Price sensitivity seems orthogonal between Spain and France for dry-cured ham.

Fig. 1. Price/quantity correlation of dry-cured ham bought by French and Spanish panellists, source: authors



In France, three categories of shoppers were identified, according to the number of packages bought. *Light buyers* are those who bought till 3 packages of dry-cured ham, *Medium buyers* are those who bought 4 or 6 packages of ham and those who bought more than six packages of ham were characterized as *Heavy buyers*. Most of the buyers could be characterized as *Light buyers* (42.4%). There were a high percentage of *Heavy buyers* (28.7%), who bought a big percentage of total purchases. The majority of the buyers bought two different products (47.3%), only few of them bought one product (9.6%). There were a considerable number of buyers who bought more than 4 different products (15.4%).

In Spain, 84% of the total number of clients only bought one specific dry cured ham category, and Serrano attracts the maximum loyalty degree. Only two categories are bought by 13.4% of the customers and only 2% of the consumers buy three categories, while only 13 customers buy up to 4 categories nobody buys from the six specified categories. The majority of consumers (71 %) did not buy more than two articles along the analysed period, and almost half of them bought one only article

(48.7%). As there is a low buying frequency it is difficult to affirm about the loyalty. However, we might think that the buying repetitions indicate assertive consumers with respect to the different categories.

While in France Serrano is considered as typical Spanish ham and faces a low purchase frequency, in Spain, this category prevails for the number of customers as well as for purchase value. The average price of this category is clearly lower than for the rest of hams, which seems to mean that price influences considerably consumers' choice, above other considerations as the region of origin, or the collective quality label. Only a minority of customers buy Iberian ham, and even a lower proportion are devoted to Iberian only. Several factors explain this outcome, but tradition is the first. Zaragoza is located in an area of white ham production and not Iberian, leading to a wider number of customers. Second, Teruel ham in particular, is also produced in the region, which implies that customers are not only used to white ham in general but also to ham produced in Teruel, in particular. And third, Iberian ham is at least 2 to 3 times more expensive than white ham, even Teruel ham with DO.

The purchase frequency is very low and also the ham expenditure throughout the period under investigation. In fact, more than half of the customers spent less than 12 €, and bought cured ham less than twice during this period. A likely explanation for this low purchase frequency in Spain can be that cured ham is a product mainly consumed outside home, in bars and restaurants. Also, it is feasible that clients do not buy ham in hypermarkets but they also buy in other retail outlets.

In both countries, highest loyalty degrees occur between the brand type categories where consumers have a low propensity to switch. The lowest propensity to switch is within the brand and price categories. This suggests that consumers stay loyal to a specific brand type and price range as well. On the other hand, Designation does not have high loyalty levels, hence this category has the lowest levels, suggesting that Designation is not an important loyalty driver with the exception of Teruel DO. Consumers do switch between DO and no DO products but they stay loyal in specific brand types and price levels. The type of brand and the price were the most important loyalty drivers. However, designation did not reach the high levels of expected loyalty and probably it is an important factor for a segment of consumers but not for the majority of them.

Tasting preferences

The preference tests were managed, in shops, in France and Spain during summer and autumn 2004. The volunteer consumers for hedonist tests were recruited among the scanned panel of consumers used to observe their purchases in order to compare sensory and actual preferences. The tasted hams were chosen among those available on the usual market places for consumers. The global appreciation of 10 samples were measured by a blind test and the information significance (origin, breed, brand, quality label) was appreciated during a second tasting (identified test) in the same session, with information written on a sticker.

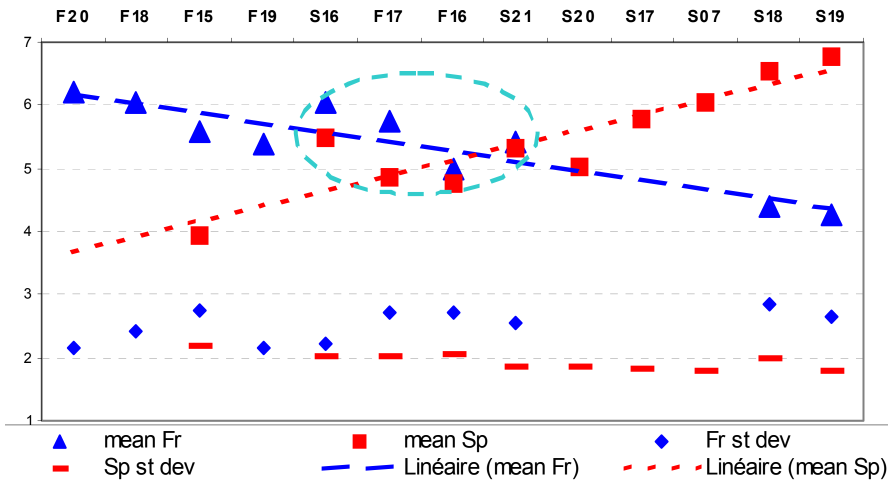
Ten samples of dry-cured hams were evaluated by each country: 6 domestic samples and 4 foreign samples, although in Spain there was 3 French hams. The hams were tested by French and Spanish consumers from the panel whose purchases were recorded through loyalty cards. Samples were selected by professionals of the supply chain according to segmentation criteria: region, breed, feeding, production scale, maturation, brand, label of origin, availability at the distributor partner of the research consortium. 7 samples were common in the two countries to analyse the cross-cultural perception of typical or conventional dry cured hams. The sampling includes, in both countries, standard and local breeds from white and black pigs, commercial and distributor brands, several regions, PDO (Protected Designation of Origin) or PGI (Protected Geographical Indication) *vs* no DO labels, as well as low and high price hams.

There are clear differences of French and Spanish consumers tastes: French consumers rank Iberian hams last while Spanish consumers rank them first. French consumers like French hams much more than Spanish consumers do. French consumers find some similarities between Spanish white hams and French ones, as they assign close scores to hams falling into both origins, while Spanish consumers clearly group the French hams together, and lower than the rest of the hams. Consumers like what they are used to eat.

The following figure clearly shows that going from most bought French dry-cured ham to the most typical Spanish ham decreases the blind hedonist preference of French consumers and increases the Spanish ones'. Standard deviation of French assessors is always higher than those of Spanish ones', indicating more scattered preferences. The dispersion of appreciation is squeezed when the product is well appreciated, for both French and Spanish hams.

Interestingly, two French and two Spanish hams reach a relative consensus within French and Spanish consumers. Three of them bring quality label, but not strictly an origin label, and all have a medium length of maturation and are made with legs from white large breed. These four dry-cured hams are not the preferred in each country but offer a good palatability for most consumers living in these regions.

Figure 2 Effect of familiarity with product on blind preferences of French and Spanish assessors, source: authors

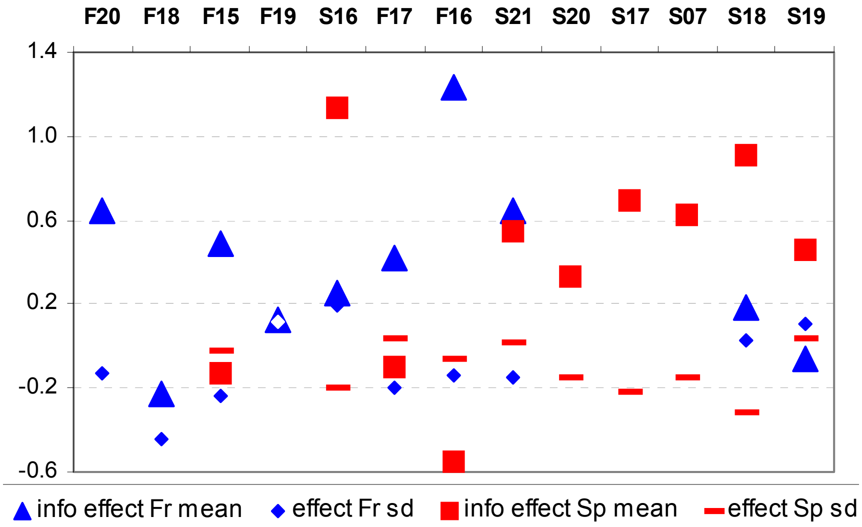


The effect of information is congruent in both country: notoriety of brand, distributor's label and region of origin (if well-known) increase the hedonist appreciation of assessors. The positive effect of PDO or PGI label is not clearly established but, in Spain, some DO hams are clearly appreciated. The effect of information reduces dispersion of consumers' responses in France and a bit less in Spain. The brand and the region effects are the most influential, while the effect of distributor branding is unequal. Strong brands ever taste better.

In any case, Spanish consumers are quite consistent, as no sharp changes of rankings occur to any of the samples, in contrast to the French experiment, where the distributor's brand jumps from the 2nd to the 7th position, and Aveyron ham, from the 8th position to the 3rd. This means that the distributor's brand has got a bad connotation in France, while this is not the case at all in Spain.

The most bought ham, commercial brand, and the local regional hams are well appreciated when identified in France, in spite of their opposite place on the prices range. Despite Iberian hams are blind preferred over the rest of white hams by Spanish consumers, Iberian hams are not purchased as much as white hams in the region of Zaragoza. Price could be an important reason: the average price for Iberian ham in the scanner database is 50 €/kg when sliced on request at the delicatessen section, but can go up even more when sold in the self service section, packed in slices. In contrast, Teruel ham price is around 21-17 €/kg, depending if it carries or not the DO label.

Figure 3. Difference of ratings between blind and identified hedonic tests from French and Spanish assessors, source: Authors



CONCLUSIONS

Results show that the dry-cured ham market can be characterized as a highly segmented market with few loyal buyers. Strong commercial brands have a great influence while small brands gain the respect of heavy and loyal consumers.

Consumer purchase behaviour towards dry-cured ham is obviously price sensitive, suggesting that consumers' preference is based on the products' price. Many consumers tend to be loyal to specific attributes of the products but what drives loyalty is not the same on what drives volume. Our results show that price is a factor which affects loyalty but mainly attracts volume especially when this is in combination to decide about promotion strategies. Designation is not such an important driver as theoretically expected. Brand type is one important loyalty driver and this might happen as the panel consisted of loyalty cards owners and there is a high proportion of them who are loyal to distributors' brands. For further research, a panel should be wise to avoid this bias.

Finally, when PDO-PGI producers believe that their hams are of superior quality, consumers are more interested by the image of the region of origin, the brand notoriety, but always choose their purchase under price considerations. This gap highlights the need for producers to undertake research and understand who the buyers are and what influence their behaviour.

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CORSICAN PIG BREED AND PDO PROJECT FOR PROCESSED MEAT: MAIN CHALLENGES ON COLLECTIVE MANAGEMENT

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SUMMARY - Local pig breed has recently been recognized and its managers are stabilizing technical choices about its genetic. Thanks to this dynamic a regional syndicate is created to be an official applicant of a PDO for Corsican processed meat. Within the application, low growth local breed is exclusively mobilized to insure a strong link with the "terroir". We show consequences of such assignation of the genetic resource on its management requirements. We purchased several interviews with main concerned actors (breeders, technicians, and animators) and participant observation during a lot of meetings (technical, professional) attended by the breed managers and the PDO applicants. Main results deal with: i) Reproductive animals' availability; to complete animals provided by breeders from the Herd Book, a breed register is supposed to be based on phenotypical traits. Providing animal on PDO farms by both the sources put in tension *breed type vs* objectives aptitudes; ii) Rules for reproductive animals management; non-breeders users of the breed are potentially not allowed to practice "intern turnover", this dependence of the breeders is questioning their own statute. Discussion underlines the role of interactions between the two collectives in designing the local breed future.

Key words: local breed, breeding stocks, projects, organization forms.

INTRODUCTION

Corsican pig breed has recently been recognized by the French CNAG (National Commission of Genetic Improvement) and its managers are stabilizing technical choices about its genetic (CASABIANCA *et al*., 2000). The regional organization in charge of this management is the first professional skill appearing in this sector. Thanks to this dynamic, a regional syndicate was created to be an official applicant of a PDO for Corsican processed meat. In this new project, a code of practices defining rules of production has to be fixed.

Within the application, low growth local breed is obviously mobilized to insure a strong link with the "terroir". In a first step, the application was based on the possibility to produce from pure breed animals and also from cross-breed. It planned to accept also two modes of finishing period feeding : chestnuts and/or acorns vs barley. In this perspective, a set of four possibilities (two genotypes and two finishing feedings) was proposed, with a special mention for animals from pure breed and with chestnuts and / ou acorns finishing feeding.

But, during the application procedure, the French institute in charge of these applications (Institut National de la Qualité et de l'Origine INAO) reduce this possibility and applicants decided to choose only the pure breed as they maintain the two finishing feedings. In this situation, new stakes around the genetic resources appears due to the fundamental role of the breed within the PDO project.

We show consequences of such assignation of the genetic resource on its management requirements and try to answer two major questions:
Which animal resource to choose in the PDO decree: *pure breed* or *breed type*?

Which rules of breeding stock management are chosen?

MATERIAL AND METHODS

First of all we purchased several interviews with main concerned actors of the two projects:

- breeders (inside or outside PDO project),
- technicians (of breed management, of PDO syndicate),
- and animators.
-

So we gather the different point of view of those actors about technical choices for the PDO project and rules of breed stock management for the breed project.

The second tool used for this study is participant observation. We could assist to a lot of meetings attended by the breed managers and the PDO applicants. By the way we participate to boards where producers discuss about political choices, but also to technical committees where technicians and animator discuss about technical choices. So we could notice the arguments from producers, technicians and animators in real time.

RESULTS

Main results of our study deal with breeding stocks availability and rules for breeding stocks management.

Which animal resource for the PDO project?

Discussion between actors was about *pure breed* and *breed type* for the PDO production. Let us begins by few definitions. In the decree n°2006/1662 of December the 21st 2006 relating to the identification and the genetic improvement of the animals: "*Breed: a whole of animals which has sufficient joint points to be able to be regarded as homogeneous by one or more groups of stockbreeders who agree on the organization of the turnover of the breed stocks and the induced exchanges, in particular at the international level*." To associate the concept of purity introduces the need of inscription of ascending (parents and large parents) within the HB (Herd Book) of the breed, and the inscription of the individual concerned. In the case of the HB for Corsican pig breed, it is necessary to know the ascending on 3 generations so that an animal can be registered within the principal section of the HB.

The concept of the *breed type* has neither legal definition nor a definition which is accepted by all. In the various animal fields, this term is employed according to several directions. In the decree of July the 2nd 2001 relating to the identification by radio frequency of the domestic carnivores: "*Breed type: the wording of the phenotype of the animal, this element of appreciation of appearance should not be interpreted as a breed within the meaning of its inscription with a herd book* ". The *breed type* then becomes a phenotypical indicator, which does not engage an official recognition. We choose this definition for the concept of the *breed type*, which corresponds to a part of the denomination of the breeds in the PDO Beaufort decree, and with the definition of what would constitute a register for the processed meat of Corsica PDO. A breed register is supposed to be based on phenotypical traits. Providing animal on PDO farms by both the sources put in tension *breed type vs* objectives aptitudes (LAMBERT-DERKIMBA, 2007).

Using animals of the *breed type* for the production of PDO makes possible increase of the number of eligible animals. But what about the competition then created around the animals of *pure breed*? In order to understand the competition between the two resources allowed (*pure breed* and *breed type*), we try to identify their differences.

Table 1. Differences to obtain the two resources

Genetic resource	Pure breed	Breed type
Identification of animals	Herd book and standard	Register and standard
Transaction costs (GUERRIEN, 2002)	Mobilize new networks	Use of former networks
Structure of the farm	New technical rules of management to ensure filiations and mating: structure investment	Former rules allow just to know the parents: no structure investment

In the case of the pure breed, producers have to invest in several kinds of tools: official identification, networks and structuring the farm. In addition, they have to pay higher price to build a stock breed because of the genetic value of animals. In the case of breed type, producers just have to follow usual rules and don't have to invest in new tools: identification is classical, no need of new networks and no need of structure investment. This situation means that producers could have the choice between 2 resources and one of the 2 is cheaper (*breed type*) than the second (*pure breed*). The risk of forsake the *pure breed* is clear.

After months of *statu-quo*, breed managers became aware of this stake and stopped the possibility for a register. As a provisional issue, the *pure breed* is today the only resource allowed by the conditions of production of the PDO. The *pure breed* is so protected by the elimination of competition.

Which consequences on the stock breed management?

This situation leads to the increasing of the demand in *pure breed* animals. All the producers who want to be in the PDO project have to provide his farm in *pure breed* animals. As a consequence, the value of the pure breed becomes higher (LANGLOIS, 1998). The question for the breed association becomes: how to manage the reproductive animals' production? Breed managers decided to keep for themselves the possibility to produce reproductive animals.

The rules for the management of the breed seem very simple:

- Breeders have the exclusivity to produce reproductive animals
- Breeders have the exclusivity to sell reproductive animals
- Producers cannot self-renew their reproductive animals

Such rules have several consequences: all the farmers in the PDO project must buy every year the turnover of their herd, because only pure breed is allowed by the project and only breeders can produce it. This creates a situation of allowance for the breeders who are sure to earn money every year from their selection work. But that means that there is some limitation in the available animals (reduction of the potential market) both for reproduction within the herds and for the PDO production.

And also non-breeders producers have to change their practices induced by the extensive system. Traditionally, pig producers in Corsica manage by themselves the reproductive animals' turnover (SABY, 1996). They choose among the female the ones better for some traits (prolificacy, growth and health), but also for some skills (exploitation of the territory and the natural resources). If reproductive animals provide by the breeders could have a better genetic value in terms of prolificacy, growth and health, what about the capacity to exploit territory? This character expected by the producers proceeds from maternal training. Such learning process is performed when the relationship between mother and young piglets is intense. So, introduction of young external female in a given batch provokes some aggressive behaviour and avoid the possibility to this learning process. As a consequence, farmers will have to manage new situations in terms of batch of animals and reproduction management. And they have to face some new difficulties due to the lack of capacities of their animals to exploit the territory.

DISCUSSION

We can underline the role of interactions between the breed managers and the PDO applicants. The members of the breed association are closed to be all included in the PDO project, the vice president of the breed association being the president of the PDO syndicate. Moreover the animator of the association is also in charge of the syndicate (half-time on each institution). In such a situation, it's difficult to make a difference between the two collectives: the borders are fuzzy. So, management rules which seemed simple put the non breeders users of the breed in dependence of the breeders, questioning their own practices and their professional skills.

During several months, we could see that the objectives of the PDO project overcome the objective of the breed project. For the PDO applicants the recognition and the management of the breed are considered as a main justification of the PDO certification. This is the reason why the idea of a register was born in spite of the risk for the breed future. So, after a synergetic period, the perspective of the PDO certification is now weakening the breed project.

But by a second time, the breed managers realised such a risk. After long debate where the former president took an important role and where each producer dared to express himself, the register project was cancelled. In such a situation, the future of the breed depends on the issue of the discussions between the two boards.

On another hand, the interactions between the collectives make possible the creation of a situation of allowance. The rules of management for the PDO (only the pure breed for the PDO) and the breed (no turnover of stock breed for breed users) seem coherent to support breed managers by giving them a key role. The situation of allowance is a consequence of this coherence.

CONCLUSIONS

The case of the Corsican pig breed questions the appropriation of the genetic resource. What is legitimacy for a PDO syndicate to appropriate such a resource? Finally, could we consider the PDO project as supporting the development of the genetic resource?

According to this case, we can assume that a breed depending only on another project such as PDO application becomes endangered for its future. The breed project must exist by itself, and, in this perspective, the convergence of the two projects could be an opportunity for both. The breed could support the PDO by reinforcing the territorial legitimacy and the PDO could support the breed by providing a market. A right balance should insure that decision-making in each of the two boards are autonomous.

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CONSUMER ATTITUDDES TOWARDS DRY-CURED IBERIAN HAM OF PROTECTED DESIGNATION OF ORIGIN GUIJUELO

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SUMMARY - Spain is the main producer and consumer country of dry-cured ham in the world. Protected Designation of Origin Guijuelo corresponds to hams manufactured from Iberian pigs, which are characterized by high levels of subcutaneous and intra-muscular fat due to the diet. Nowadays, the consumer's preferences have a tendency to attach importance to affective aspects, such as regional origin, what affects consumer purchase behaviour. For that, it is necessary to know if correct information of consumers, helps to them, to distinguish between products with the same origin, but with different quality. So, triangle tests were employed to determine if consumers could distinguish between samples of Iberian ham "Bellota" or "Recebo". The results showed that the consumers were able to found differences between samples. The brightness, colour and odour intensity and taste were the main parameters that allowed differentiating the samples. Therefore, information about the product helps the consumer to choose among Iberian ham options.

Key words: dry-cured Iberian ham, sensorial, consumers.

INTRODUCTION

Since 1985, origin certification schemes have been in place in the cured ham sector in order to avoid the misuse of origin claims, to inform consumers about the true origin of the products they buy, and guarantee the compliance with specific production rules and quality controls. Currently, a total of four Protected Designations of Origin (PDO) correspond to Iberian breed have achieved European protection: PDO Guijuelo, PDO Dehesa de Extremadura, PDO Huelva and PDO Los Pedroches.

PDO Guijuelo corresponds to hams manufactured from pigs of Iberian breed, which are characterized by high levels of subcutaneous and intra-muscular fat due to the diet. A correct identification of this product, taking into account the fed, is: (i) "Bellota" Iberian ham (from an Iberian pig fed on a "montanera" -diet of acorns and natural grasses-) and (ii) "Recebo" Iberian ham (from an Iberian pig that has been fed on a mixture of acorns, grasses and fodder).

Resano *et al.* (2007) pointed out the geographical origin affects consumer preferences and purchasing decisions first, indirectly as a quality cue and second, directly through the sense of belonging to the region of production and other affective aspects, or as a way to comply with ethical or normative rules through purchasing behaviour. However, these affective aspects could confuse to consumer in their purchase behaviour. For that, it is necessary to know if the information of consumers helps to them to distinguish between similar products. In this study, the aim was to know if the correct information of consumers helps to them to distinguish between products with the same origin, but with different quality ("Bellota" Iberian ham and "Recebo" Iberian ham).

MATERIAL AND METHODS

In order to know if consumers could distinguish between Iberian ham with different quality, consumers carried out triangle tests (ISO 4120:2004).These tests are extensively used in food analysis to determine if differences between two products can be detected from a sensory point of view.

Consumer's tests were carried out in a special room with tables in the same direction, in order to avoid communication between participants, with white fluorescent lighting and kept a temperature of 21±2ºC. Tests were carried out at a location of "Castilla y León" different of Guijuelo.

Consumer's panel consisted of a variety of students, housewives and staff of different organizations with ages ranging from 20 to 65 years and habitual consumers of dry-cured ham. Sessions were organized in the morning and the afternoon along four days. The two first days consumers evaluated "Bellota" and "Recebo" Iberian ham samples in blind information conditions (they only know that the samples was Iberian ham from PDO Guijuelo). The other two days, information about the characteristics of the samples to evaluate was done to the participants previously to the analysis (information conditions).

Samples, consisting of "Bellota" and "Recebo" Iberian ham slices of 1.5 mm of thickness, were obtained with a slicing machine and served on plastic plates to participants. Slicing was carried out in order to obtain slices containing exactly the same muscles. Three coded samples were presented to each panellist, and each panellist was asked to pick out which sample they feel different from the other two. Samples were presented in a random order and in a balanced presentation. These had kept at environmental conditions (21±2ºC) for at least half an hour before the tasting. Unsalted bread and water were available and recommended in order to clean the palate between successive samples.

The statistical interpretation of the test was based on the binomial law with p=1/3 (null hypothesis, no differences between the two tested products). When the number of correct answers of a triangle test equals or exceeds the critical value compatible at a given probability level, the null hypothesis is rejected and significant differences between samples are concluded. The critical value was obtained from the Roessler *et al.* (1948) tables.

RESULTS AND DISCUSSION

The characteristics of consumer's panel are show in the Table 1. In general, about 50% of consumers were women and the other 50% were men. The number of consumers in each session was ranged between 38-58 participants.

Table 1. Characteristics of consumers' panel.

	Blind information conditions			Information conditions		
Session	Number of consumers	Gender		Number of consumers	Gender	
		% Women	% Men		% Women	% Men
Morning	48	53.1	46.9	54	44.5	55.5
Morning	41	59.4	40.6	58	46.6	53.4
Afternoon	47	54.5	45.5	58	56.9	43.1
Afternoon	38	50.0	50.0	51	39.6	60.4

Respect to the results of triangle tests (Tables 2 and 3), consumers with blind information conditions (Table 2) distinguished ($p<0.01$) between "Recebo" Iberian ham and "Bellota" Iberian ham in the first session in the morning; however, in the others sessions, consumers did not found differences.

Table 2. Results of triangle tests carried out with consumers in blind information conditions.

Session	Number of consumers	Correct answers		
		Total	Women	Men
Morning	48	35***	19***	16***
Morning	41	18	13*	5
Afternoon	47	21	12	9
Afternoon	38	15	6	9

*p<0.05; **p<0.01; ***p<0.001.

The results obtained from consumers in information conditions (Table 3) were very different. In all sessions carried out consumers distinguished between "Recebo" Iberian ham and "Bellota" Iberian ham. But, it must be emphasized that probability level was different between the session organised in the morning ($p<0.001$) and the afternoon ($p<0.01$ or $p<0.05$). Besides, different significant level was found when consumers were women or men, being women who distinguished between hams with different quality with a lesser probability level.

In general, the consumers made comments about the colour, brightness, odour and taste of the samples, as the main attributes in theirs decisions.

Table 3. Results of triangle tests carried out with consumers in information conditions.

Session	**Number of consumers**	**Correct answers**		
		Total	**Women**	**Men**
Morning	54	34***	13*	21***
Morning	58	37***	17**	20***
Afternoon	58	29**	16	13*
Afternoon	51	25*	11	14

*p<0.05; **p<0.01; ***p<0.001.

CONCLUSIONS

Therefore, information about the product has an effect on sensory valuations and helps the consumer to choose among Iberian ham options.

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BEHAVIOUR OF CONSUMERS TOWARDS THE CONSUMPTION OF PORTUGUESE TRADITIONAL SAUSAGES

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SUMMARY - Traditional sausages are usually safe due to its intrinsic parameters. The handling practices and consumption habits might increase the risk of contamination or pathogen overgrowth, namely by cross contamination or temperature abuse. The purpose of the present research was to evaluate the self-reported behaviour of traditional dry sausage consumers about safe practices in purchasing and home handling. Consumers (180) of dry sausages completed a questionnaire on: socio-demographics, consumption habits and handling practices. A variable *Risk Level* (RL) was established considering the amounts consumed, package, temperature abuse, consumption by children and elderly people. Consumers were grouped in two categories: lower and higher RL. Comparison of proportions was performed using Chi-square test and multiple logistic regression. It was observed that men presented higher risky behaviour (OR=2.487, p=0.010). Consumers working in administration or trade are less risky than others (OR=0.395, p=0.040). Regular consumer presents safer behaviours (OR=0.409, p=0.006).

Key Words: Consumer, behaviour, dry sausages, traditional products

INTRODUCTION

Food safety is an important concern for the professionals in the food and food services sectors. Meat and meat products are frequently associated to foodborne outbreaks, mainly associated to *Salmonella* spp., *Staphylococcus aureus*, *Clostridium botulinum* and *Listeria monocytogenes*. In the period 1998-2001, Rocourt *et al*. (2003) reported foodborne outbreaks in OCDE countries associated to meat and meat products as responsible for 5 to 30% of the reported cases. However, when these incidents were studied in detail, it was clarified that the most part was related to raw meat prepared at restaurants or at home, or, to cooked meat products – insufficiently cooked, or cross contaminated.

Dry cured products are rarely responsible for foodborne diseases, once its intrinsic parameters are enough to avoid pathogens growth to levels infectious to immunocompetent population. Even with centuries of experience manufacturing these products, it is still observed punctually, foodborne outbreaks related to fermented sausages and other non-heated ready-to-eat meat products (Moore, 2004).

Considering the data from the 8th report (1999-2000) of WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe, the data from Germany, Belgium and Italy (the only with detailed information about the type of meat product responsible for the outbreak) the proportion of dry cured meat products is lower than 1%, suggesting that these products are the safest among the meat products.

Nonetheless the intrinsic safety of dry cured sausages, the handling practices used by consumers might increase the risk of contamination or pathogen overgrowth, namely by cross contamination, temperature abuse or other incorrect practices (Talon *et al*, 2007). Moreover, consumers frequently believe that foodborne illness is a minor sickness and that this illness usually results from eating a contaminated food in a restaurant (Medeiros *et al.* 2001). These beliefs associated to a lack of knowledge of food preparers at home, could result in a serious increase in the risk of food borne disease associated with foods in general (Griffith *et al*., 1998) and with dry cured meat products in particular.

The purpose of the present research was to evaluate the attitude of dry-cured sausage consumers about safe practices in purchase and home handling.

MATERIAL AND METHODS

Population and instrument for data collection

The study involved 180 consumers of dry cured products from different regions of Portugal. Participants completed the structured questionnaires, covering aspects on consumers socio-demographics (age, sex, number of children and adults at home, region of origin, residence and residence in childhood, education level, job category, sector of activity), on consumption habits of traditional dry sausages (regularity and place of purchasing, number of sausages purchased/month, size of the sausages and of the slices, amount/day/person consumed, eating with/without casing, moment of the day/meal; consumed raw or after cooking, which consumers: adults, children, elderly, season of consumption) and on handling dry sausage habits (packaging at purchase, at home storage before and after beginning, temperature and length of storage, started sausages stored at home whole or sliced). All the questions were closed with several response possibilities previewed.

Variables

Risk Level (RL) Was established considering the occurrence of behaviours considered the more risky among the previewed ones: High amount/day/person consumed, eating with casing, do not use any package on purchasing, at home storage (whole or started one), the storage is made in the kitchen, with no special attention to the temperature; consumes preferentially at Summer, stores the sausages home for the long period, stores the stared sausage already sliced. Considering these practices consumers were grouped in two categories (lower and higher risk level) with similar frequencies.

Statistical analysis

The data were analyzed using SPSS software (version 12.0, 2003; SPSS, Inc, Chicago, IL). Statistical significance was set at a p value less than 0.05. Comparison of proportions was performed using *Chi-square* test (X^2), with *Yate's* correction when applicable.
Multiple logistic regression analysis was performed using the forward likelihood ratio method, introducing in the model variables with statistical significance in the univariate analysis. Adjusted *Odds ratio* and 95% confidence intervals were calculated.

RESULTS AND DISCUSSION

The main characteristics of the consumers are presented in Table 1. The consumers were mainly women (69%), with a high education level (62% secondary level or higher). They work in the administration and trade (83%) and live in country side or small province towns. They were almost equally distributed by the three age categories.

It was observed that those consumers with higher risk behaviour ($p<0.05$) are male and individuals working in agriculture and industry. No effect was observed of Education level, age or residence area (rural or urban), in the distribution by the two risk level categories.

Table 2 shows the results of multiple logistic regression analysis. It was observed that variables maintained the statistical significance revealed in univariate analysis, showing the same tendency. It was observed that men presented a higher risky behaviour than women (OR=2.487, p=0.010). Those referred they eat a high amount/day/person of dry-cured sausage with casing; they do not use any package on purchasing or at home storage (whole or started one). They also referred that the storage is made mainly in the kitchen, with no special attention to the temperature (even in the warmer months). The sausages are usually stored at home for a long period.

Probably due to urban behaviour of consumption or access to information, respondents working in administration or trade are less risky than those from agriculture and industry (OR=0.395, p=0.040).

Those individuals having regular habits of consumption of dry-cured sausages presented safer behaviours than the unusual ones (OR=0.409, p=0.006).

Table 1. Proportion of dry-cured sausage consumers presenting self-reported behaviours (low and high risk) as a function of socio-demographic characteristics [n (% in the category)].

Variables	**Lower Risk level 90**	**Higher Risk level n = 90**	χ^2;*p*
Age group, years			
≤ 30	37 (56.9)	28 (43.1)	3.455; 0.178
31 - 50	30 (41,7)	42 (58.3)	
≥ 51	23 (53.5)	20 (46.5)	
Sex			
female	70(56.0)	55 (44.0)	5.132; 0.023
male	20 (36.4)	35 (63.6)	
Education level			
≤ 2nd cycle (6 years)	19 (50.0)	19 (50.0)	0.031; 0.984
3rd cycle (9 years)	13 (48.1)	14 (51.9)	
≥ Secondary	56 (50.0)	56 (50.0)	
Sector or activity			
agriculture and industry	9 (29.0)	22 (71.0)	5.612; 0.018
administration or trade	81 (54.4)	68 (45.6)	
Residence area			
Metropolitan	34 (46.6)	39 (53.4)	0.369; 0.544
Country/Small city	56 (52.3)	51 (47.7)	
Regularity of consumption			
irregular	37 (38.9)	58 (61.1)	9.030; 0.003
regular	52 (62.7)	31 (37.3)	

Table 2. Results of logistic regression for Risk Level (lower and higher) considering consumer's sex, sector of activity and regularity of consumption as predictors.

Variable	Beta	SE[1]	p	OR	95% CI[2]
Sex					
female				1*	
male	0.911	0.352	0.010	2.487	1.247-4.958
Sector or activity					
agriculture and industry				1*	
administration or trade	-0.929	0.452	0.040	0.395	0.163-0.959
Regularity of consumption					
irregular				1*	
regular	-0.895	0.324	0.06	0.409	0.217-0.771

[1] SE. standard error; [2] CI Confidence Interval; * reference class

CONCLUSIONS

The self-reported behaviour in the purchasing and consumption of traditional dry cured sausages is influenced by consumers' characteristics. These differences in the behaviour are important mainly to known the higher risk level groups, viewing the conception of educational/training strategies to improve safety of these consumers.

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DIVERSITY IN PIG FARMERS' LOGIC IN CORSICA
APPROACH OF THE DIVERSITY OF FARMERS' LOGICS

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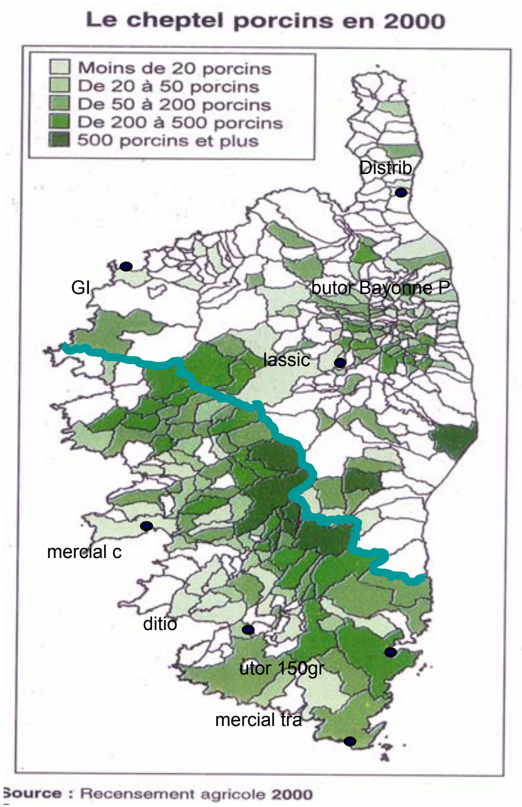
SUMMARY - Pig farmers in Corsica show a specialisation including farm transformation into pork cuts and farm sales. They reflect however differently on their perspectives. Our objective is to represent the diversity of the rationalities of the Corsican pig farmers, for which we use the *farming styles* approach. A field survey of semi-structured interviews was held with 20 breeder-processor pig producers and six experts. The farmers were chosen for their location, their expressivity and their position toward a PDO-project. The interviews were transcribed and analysed by a principle factor analysis (in SPSS). The study shows a regional style of farming: an *eco-cultural farming system*, with a logic based on seasonal production and transformation, and subtle, micro-regional distinctions. Within this style two dimensions explain the farmers' diversity: (i) the orientation on collective action, focussed specifically on the PDO / herd book project, and (ii) the incorporation of technology in the farming system.

Key words: Corsica, pig, quality, farming styles, collective action

INTRODUCTION

Pig farming traditions in Corsica have developed into a specialisation of breeding and on-farm transformation of pork cuts and farm sales. Pigs are bred in a seasonal system, fed on pasturelands and finished in mast forests (chestnuts and oak). Until recently, the pig farmers were not focussed on production organisation and institutionalisation. The management of the pig population has now become a major issue for the dynamic development of the Corsican pig production and local pork products (Casabianca *et al.*, 2000). In the development process of a *Protected Designation of Origin* (PDO) for Corsican dry cured ham, the pig farmers were forced to choose for the standardization of the genetic breed or the feed supplementation (or both). The representatives of the breeder-processors involved with the syndicate for the PDO choose to concentrate on the standardization of the breed. Since then the issue of social control over the pig breed is a major issue among the Corsican pig breeders. The question has emerged whether the development of a herd book should be fully subjected to the development of the PDO for pork products, or whether it should have authenticity in its own right (Lambert-Derkimba, 2007).

Figure 1. Swine herd density in Corsica



The foundation of an elite group (association) for the creation of management scheme for defining the breed engaged several problems. Animals bred according to the schematic criteria did not represent the local idea of the breed. A subgroup of breeders, well implemented within the regional professionals, put forward management efforts to the preoccupations of the entirety of pig farmers. Thus these breeder-selectors are the prime professionals to engage in the development and to offer their exploitations as sites for the control of reproductive animals that belong to the local breed corresponding to the criteria. Another required characteristic for the herd book consists of keeping the animals according to the production systems in conformation with the logic of extensive farming, in which the animals exteriorise certain rustic abilities (Casabianca *et al.*, 2000).

Traditional pig farming is still found in all mountain areas in Corsica, with differences depending on geographical, demographical, climatic and micro-regional circumstances (Casabianca, 2004), (Figure 1). The globalisation of agricultural knowledge and technology, and the differentiation of intermediate markets have created a new frame of reference for farming styles (Ploeg, van der 2003). The research methodology was extended and specified, and applied to pig farming by Commandeur (2003). This study is a further elaboration of these principles.

The aim of this study is to identify the options for strategic interventions in the institutionalisation processes. We conducted a sociological survey among the Corsican pig farmers in 2004-2005; just before the National Committee officially recognized the Corsican *Nustrale* breed in January 2006. Several of the farmers in this study were involved with the organisations for the standardization of the breed and the expected certification of the PDO, others kept aside, pursuing their individual plans and projects or involving themselves with alternative organisations. The principal supposition of this research is that the various reflections of the pig farmers on their perspectives are anchored in different logics. Despite individual differences, the hypothesis is that the rational foundation of an approach is an identifiable logic with legitimacy in its own right, which goes beyond the individual expression. The first objective of this study is to abstract (*stylise*) the contrasting farmers' logics among Corsican pig farmers within and outside the process of institute formation and to identify the dimensional aspects that structure the frame of diversity: to identify diversity in *styles of farming*. A subsequent objective is to improve the interpretation of scientific and technical data in the context of uncertain perspectives for the pig farmers and the sustainability of their profession.

MATERIAL AND METHODS

Before starting the semi-structured interviews with the pig farmers, six explorative interviews were done with experts, both in research and in technical service extension. Based on these interviews the themes were identified, which we addressed in the subsequent interviews with the pig farmers.
Next 20 semi-structured in-depth interviews were held in 2004-2005 with pig farmers (19 cases of men, couples, or families; one single woman) who were known to vary in farming locations and conditions, as well as in their perceptions of the profession. All interviews were recorded and transcribed wordily in text documents. The initial step in the analysed was done qualitatively in quest for elements that represented contrasts in farmers' perceptions of conflicting field situations.

Questionnaire for semi-structured interviews

The questionnaire for the semi-structured interviews was outlined from the viewpoint that interview topics should cover issues that are under the direct control of the pig farmer. It resulted in merely functional discussions about the on-farm management activities for pig breeding and pork processing, the farmer's motivation for these activities and the farmer's perceptions of how and when these activities should be done.

The interviews with the farmers covered the following topics:

a) technical information about the farmer, family and village life, the farm location, the farm size and breeding productivity, management features, meat processing capacity, and implication in organisation structures

b) passion for the profession of pig breeder and pork processor, other farming and non-farming occupations and village life in the Corsican mountains

c) the qualities of a good reproductive animal: sows and boars

d) perceptions of 'quality' in housing and management practices, animal feeding, animal breed and selection for breeding or cross breeding, hygiene in slaughtering, transformation and pork processing, and final product qualities and taste

e) animal health control, welfare and manipulations (castration, nose rings, transport, etc.)

f) seasonal and environmental management; animal feeding, agreements with land owners, tourism, land erosion and landscape conservation

g) investments and specific efforts to arrive at the desired qualities in practices and products

h) work organisation, in particular concerning the slaughter and processing period in winter

i) sources of information, technical assistance and implications in organisations and institutional structures

The interviews were accompanied by taking (more than 1000) photographs. Selected photos were categorised in fifteen image themes and later assembled in collage poster designs. At the end of 2005 a slides presentation was made for the interviewees and experts to provoke a debate in a conference meeting about the sustainability of the profession and the conflicting field situations.

Subsequently the interview texts were analysed qualitatively for the functional modalities in the farmers' responses. The modalities were codified and the entirety of codified information was analysed quantitatively in a Principal Component Factor Analysis (PCFA) using the software of the Statistical Package for Social Studies (SPSS). The individual correlation scores of the interviewed pig farmers to the first principal component were evaluated in a Hierarchical Cluster Analysis (HCA). The farmers were clustered in coherent groups. The features of each group were reassembled in stylised portraits of dominant farmers' logics.

RESULTS

Based on the modalities in the farmers' responses and supported by the photographic categorisation *the regional style of pig farming* was identified. This regional farming style is based on traditional knowledge and characterised by specialized livestock farms (pigs and other), the local *Nustrale* breed or crossbreds, rustic animals, pigs in liberty with simple housing facilities, breeder-processor farmers, labour demanding and seasons based farming practices, and small pig herd sizes, related to the high labour demand for seasonal slaughter, transformation and pork processing in the mountains, and the limited concession rights for autumn pasturing in the forests.

Analysis of farming style and farmers' logics

The PCFA and subsequent HCA resulted in four stylized portraits of farmers' logic; four *stylised modes of farming within the regional style*. Two dimensions were specified as reference for the contrasts among the farmers' logics:

(i) *objective aspects* (infrastructure, climate, demography, installation period, family structure, age, etc)

(ii) *motivations for collaboration.* However, only the latter dimension served as our discriminator for the identification of the four style modes (Table 1).

Table 1. Four style modes within the regional style of farming

Collaboration	***Agreement***	***Club***	***Assistance***	***Contract***
Drive	***Tradition***	***Opportunity***	***Norm***	***Marketing***
Approach of the profession	Incorporate business knowledge in tradition	Adapt the traditions to business opportunities		
Perception of qualification	Incorporate technology in traditional system			Adapt the production system to technological opportunities
Attitude to production and products standards			standardize production and products; reorient on markets	Apply market standards for adapting production methods and products

The four style modes represented by the motivations for collaboration coincided to an extent with different groups of farmers in objective terms: geographical location (climate, infrastructure, and landscape design), period of installation, perspective for succession, etcetera. The assemblage of all aspects contributed to the differences in motivations for collaboration, which is featured by the way the farmers express themselves (Table 2). The four style modes contrasted with each other in reference to three factors: (i) Approach of the profession, (ii) Perception of qualification, and (iii) Attitude to production and products standards. They showed contrasting approaches to incorporation of synthetic knowledge (efficiency management), new technologies and sales markets in their farming system. The ones that were most driven by *professional traditions* were very selective in developing efficiency and technologies. They are interested in *agreements* for herd book standardization and PDO certification. The ones driven by *opportunity* had the same reluctance for efficiency but less for technologies. They were interested to have a *club* with their peers. The ones that were driven by *marketing* reversed the view on their business from the viewpoint of their consumers' demands. They seek marketing standardization and appeal to the demand for market quantity by seeking *production contracts* with their peers for clustering sales. The ones that (still) depended on their peers for *practical assistance* were orientated on their peers to follow their *normative habits*. They supported the development of s*tandard norms* for production methods and product labels, but they were not very outspoken about what they should be.

Table 2. Examples of expressions related to the four style modes within the regional style of farming

IDENTITY	***EXAMPLES OF THE FOUR STYLE MODES***	***CONDITIONS***
Agreement	*Incorporate adapted technologies in the basic system and traditions*	*Historical installations:*
organisation	Pigs for revenues and the ability to live in the village	*before 1992*
Nustrale	We have tried other breeds: a failure	*South Corsica*
PDO	We work like our ancestors (father / grandmother)	*Potential for*
succession		
Association	*Adapt the old way of life to interesting new opportunities*	*Historical installations:*
share	Autumn transhumance, but chestnut orchards degrade	*before 1992*
management	We respect traditions (moon) but we compromise with modernity	*Upper Corsica*
ideas	The profession and the village life is difficult and hard	*Succession inexpedient*
Assistance	*Adapt old production methods and products to meet the standards*	*Recent installations:*
exchange	Passion for the entire, the pigs and the rural life	*since 1995*
technical	It demands skills to arrive at a good (liver) sausage	*South Corsica*
skills / aid	The (new) clients should tell us what they think of our products	
Contract	*Incorporated efficiency technologies and adapt the standards*	*Recent installations:*
supply	The aim is 8 piglets per sow / year; among others: I feed my pigs	*since 1995*
and sales	Crossbreds give a better profit	*Upper Corsica*
cooperation	For the transformation we have associates / salaries / friends	

Debate with interviewees and experts

The debate with the pig farmers and experts was featured by topics that put their views outwards and forwards: environment, landscape, animal welfare, perceptions of society, clients and tourists, organising perspectives, and taking initiatives. A large number of interviewees in the survey showed up for participation in the debate, despite known differences of opinions. The debate was vivid and lively, open and respectful to each others' opinions; the shared concern for the perspectives of their profession was more deeply expressed than the motivation for confirming mutual disagreements.

The debate in the conference meeting was directed towards the following topics / - main responses:

1. *How to limit straying pigs and train them to stay on an established track?*
   - they should enclose the garbage dumping grounds
   - put nose rings when pasturing on bog lands and other delicate soils
   - prevent straying from hunger in the dry season
   - indicate their tracks to the leading sows

2. *How to achieve a better integration of pig farming with the landscape by permitting recuperation of soils?*
   - there is severe lack of recourses for investments in enclosures
   - the government at different levels is not preoccupied with our needs
   - the PDO will oblige us to take certain measures / - one does not always do what he should
   - pig farmers are seldom land owners; land erosion all depends on animal pressure

3. *How to conciliate the nature of pigs and the labour environment of the pig farmer?*
   - castrating females should be done under sedation / - animal suffering is a relative issue
   - there is contradiction about the nose ring: it is both a problem and an advantage
   - there is contradiction about slaughtering conditions and the use of slaughter houses

4. *Can one live a life anchored in traditions and also comply with the desires of modern life?*
   - it is a societal phenomenon that the villages are getting empty
   - the PDO certification should reinforce the motivation for pig breeding
   - the people also want a life aside farming; they don't want their children to succeed

5. *Will the recognition of the Nustrale breed and the protection of products by a PDO be sufficient for the construction of perspectives?*
   - the real question is: can we earn a living; an acceptable life with our family?
   - the constraint is no volume increase; sales prices with PDO should multiply by three
   - the debate is held 30 years too late; the desertification has already taken place

6. *What other initiatives should be developed?*
   - slaughtering in slaughterhouses is objected; people are not (yet) habituated
   - for maintaining the link pigs-and-chestnuts the chestnut forests should be maintained
   - acorns are underestimated as pig feed recourse
   - whether we will have perspective is a question for the collective organisation too
   - we should valorise the profession in the eyes of society and of the tourists

DISCUSSION AND CONCLUSIONS

The results of this study are original and incomparable to the situations studied previously in the Netherlands, French Brittany and Midi Pyrenees (Commandeur, 2003; ibid. *et al*., 2006; 2007), because they reveal a typical regional farming style based on endogenous traditions. They remind of the regional farming styles in the 1950s in Netherlands where local normative perceptions determined the farmers' activities, which were drawn into the conceptual framework of styles (Hofstee, 1985). In this case however, we face a special kind of a regional farming style, featuring fierce reluctance to incorporate external (synthetic) knowledge from the global scientific domain. The farming activities are driven by a strong cultural identity and local knowledge transmitted endogenously; often even within families. The breeder-processors continue to pursue for perspectives with seasonally based business practices. They are focussed on *eco-cultural* sustainable interrelations with the natural environment and on transmitting the regional (local, family) identity into specific quality pork products.

Technical experts are concentrated on techno-economic aspects, because that is what they are expected in the discourse of their jobs. The Corsican pig farmers however, tend to relate spontaneously more to specific local and socio-economic conditions. They also reflect this preoccupation in their breeding principles and farming practices (Casabianca *et al.,* 1993). The three textboxes in the article demonstrate a scope transformation: from interviews with a technical approach in the first box, passing the analysis and synthesis of the farmers' attitudes and logics, to a lively debate that emphasises serious concerns about the social and socio-economic aspects. So the conduct of the study shows how researcher (although initially sharing the expert position) achieved to identify the pig farmers problems in the scope of their space of information. In Corsica the technicians are now facing the next step: how to incorporate the identified problems in the formal interactions with the pig farmers? It will not just require a different armamentarium for intervention, but a fundamental reconsideration of what their job is about.

The four style modes were notidentified in reference to the dimensions *scale and intensity* as was conceptualized by Van der Ploeg (Ploeg van der & Long, 1994), although the framework of *market and technology* did apply in a special form. In this study the dimension *market* is transformed into the basic motivations of farmers to search for mutual collaboration. The driving force is not a market demand nor a desire to create or open markets for putting an offer; it is directly driven by the process of specialisation and professionalisation of the pig farmers. The contrast referring to the dimensions of technology reveal the struggle on how to deal with technology in a farming system that legitimises its perspectives by tradition. In this frame the quest for herd book standardization and PDO certification seems only one of the motives to interrelate. THat is however, the only subject that the farmers formally discuss in organised meetings. Other issues are addressed under the cover of this formal topic. In 2007, mutual conflicts among the pig farmers have emerged, concerting the contrasts of the style modes, and paralysing the advancement towards the PDO. The observations confirm the validity of the analysis of this study, as well as they reflect that the technicians and experts have not yet found a different way to approach the farmers' problems in a different way. Therefore the next research step will be the analysis of the farmers' support system from the perspective of the farmers' needs.

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CONFERENCE ON THE "SUINO NERO SICILIANO (NERO SICILIANO PIG)"

The role of National Focal Point

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SUMMARY – In 1994 a FAO (Food and Agriculture Organization) - CGRFA (Commission on Genetic Resources for Food and Agriculture) agreement led to the definition of the GS.FAO.MANGR (Global Strategy for Management of Animal Genetic Resources), with the establishment of NFPs (National Focal Points), RFPs (Regional Focal Points) and three permanent Woking Groups. In the same year, the Italian Government accredited, near FAO, the ConSDABI (Consortium for the Experimentation, Dissemination and Application of Innovative Biotechniques) as NFP. Some of the main functions of NFP are: (i) inventory and monitoring of the national animal genetic resources (European Farm Animal biodiversity Information System, EFABIS); (ii) drawing up of guidelines for ('*in situ*' and '*extra situ'*) conservation of the aforesaid resources; (iii) *advicing* the interested (private and public) bodies about optimal procedures for genetic characterization of autochthonous genetic types (AGTs) and/or ancient autochthonous genetic types (AAGTs) at risk of extinction and/or abandonment; (iv) promotion of sustainable development; (v) *utilization* of autochthonous animal genetic resources, as irreplaceable source, to obtain a "*local typified labeled product*" (LTLP); and (vi) education of the population to the biodiversity safeguard.

Key words – biodiversity, safeguard, LTLP, complexity.

HISTORICAL OUTLINES

On April 17th 1990, the Ministry of Agriculture and Forestry (MAF), currently Ministry of Agricultural, Food and Forestry Policies (Mipaaf), with the support of Italian Farmers Association (AIA), Circello Commune (Province of Benevento) established the National Centre for the Safeguard of the Animal Germplasm at Risk of Extinction (CESGAVE).

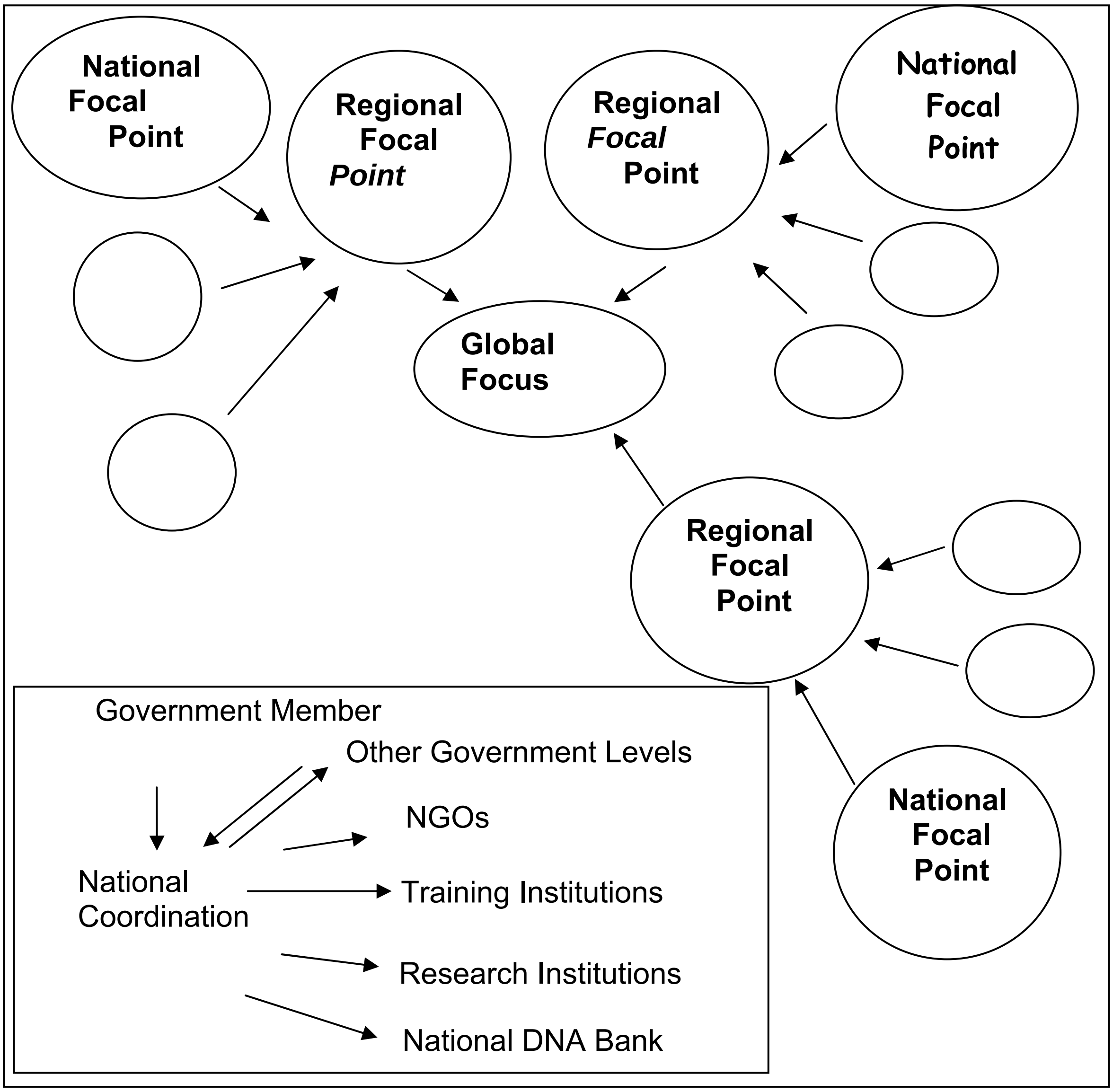
In 1992, this Centre was absorbed by Consortium for the Experimentation, Dissemination and Application of Innovative Biotecniques (ConSDABI), whose founder members are:
Italian Farmers Association (AIA), Chamber of Commerce Industry Handicraft and Agriculture of Benevento (CCIAA), Circello Commune.

Currently, ConSDABI is composed by: (i) ordinary members: Italian Farmers Association (AIA), Circello Commune, CCIAA of Benevento, Province of Benevento; and (ii) supporting members: National Italian Friesian Farmers Association and prof. Donato Matassino.

In 1994, FAO, in the context of the *Global Strategy for Management of Animal Genetic Resources* (GS.FAO.MANGR), set up: (i) CGRFA (*Commission on Genetic Resources for Food and Agriculture)*; (ii) NFP (*National Focal Point*); and RFP (*Regional Focal Point*).

In the figure 1 the organization of GS.FAO.MANGR is summarised.

Figure 1. Organization of the *Global Strategy for Management of Animal Genetic Resources* (GS.FAO.MANGR)



In 1994, Italian Government accredited ConSDABI as *Italian National Focal Point* (I.NFP) near FAO.

In 1995, DAD-IS (*Domestic Animal Diversity - Information System*) of FAO was established and in 2007 DAD-IS was supplemented by EFABIS (*European Farm Animal Biodiverity Information System*).
In 1997, during the Vienna EAAP (*European Association for Animal Production*) /FAO Meeting, the National Coordinators expressed the need to establish a *Regional Focal Point* for Europe (ERFP, *European Regional focal Point*); this ERFP became operative in 2001 with the following main activities: (i) promotion of technical cooperation based on coordinated conservation plans of animal genetic resources, with particular reference to transboundary regional genetic types of zootechnical interest; (ii) mobilization of financial resources for European projects; and (iii) communication among the various NFPs within Europe and among RFPs at global level.

Functions of NFP – FAO

The main functions of carried out by a *National Focal Point,* on FAO's mandate, are:
(i) *inventory and monitoring* of national animal genetic resources at risk of extinction and/or abandonment within EFABIS*;*

(ii) *drawing up of guidelines* for *('in situ'* and *'extra situ') conservation* of the animal genetic resources at risk of extinction;

(iii) *advicing* the interested (private and public) bodies about optimal procedures for genetic characterization of autochthonous genetic types (AGTs) and/or ancient autochthonous genetic types (AAGTs) at risk of extinction;

(iv) *evaluation* of the effects of agricultural practices, of agriecosystems and social activities on (a) animal biodiversity at risk of extinction and/or abandonment; (b) current and potential sustainability of animal genetic resource suggesting peculiar models for the valorisation of micro-agro-ecosystems (micro-bioterritories) otherwise neglected;

(v) *collaboration* with public institutions, research and experimental institutes,public and private bodies, universities, farmers;

(vi) *periodical drawing up* of a report on the national animal genetic resources *status*, in collaboration with the agricultural policy Ministry;

(vii) *utilization* of autochthonous animal genetic resources, since that it is an irreplaceable source for obtaining a "*local typified labeled product*" (LTLP), characterised through *'omic'* science approach, in its content in biomolecules of '*nutritional*', *'extranutritional'* and *'health'* value, aimed at improving welfare and well being (Fig. 1) of the consumer considered as "co-producer".

In 1980, the International Union for Conservation of Nature (IUCN), in collaboration with the World Wildlife Fund (WWF) and FAO underlined the strategic importance of safeguarding the variability of the living forms, defining their maintenance as: *"the management of the human interactions with the variety of forms of life and ecosystems to maximize benefits that furnish today and to maintain their potential to face the needs and the aspirations of the future generations"*; (viii) yearly, *drawing up* of *a report*, presented to Mipaaf, on the *status* of Italian animal genetic resources at risk of extinction, distinctly for genetic type (GT)/ AGT/ AAGT within cattle, equids, goat, pig and sheep species; and (ix) *partecipating* in: regular sessions every two years (months of March, June, September, October, November and December), extraordinary sessions and *meetings inter* – sessions organized by CGRFA (*Commission on Genetic Resources for Food and Agriculture*).

Safeguard of biodiversity

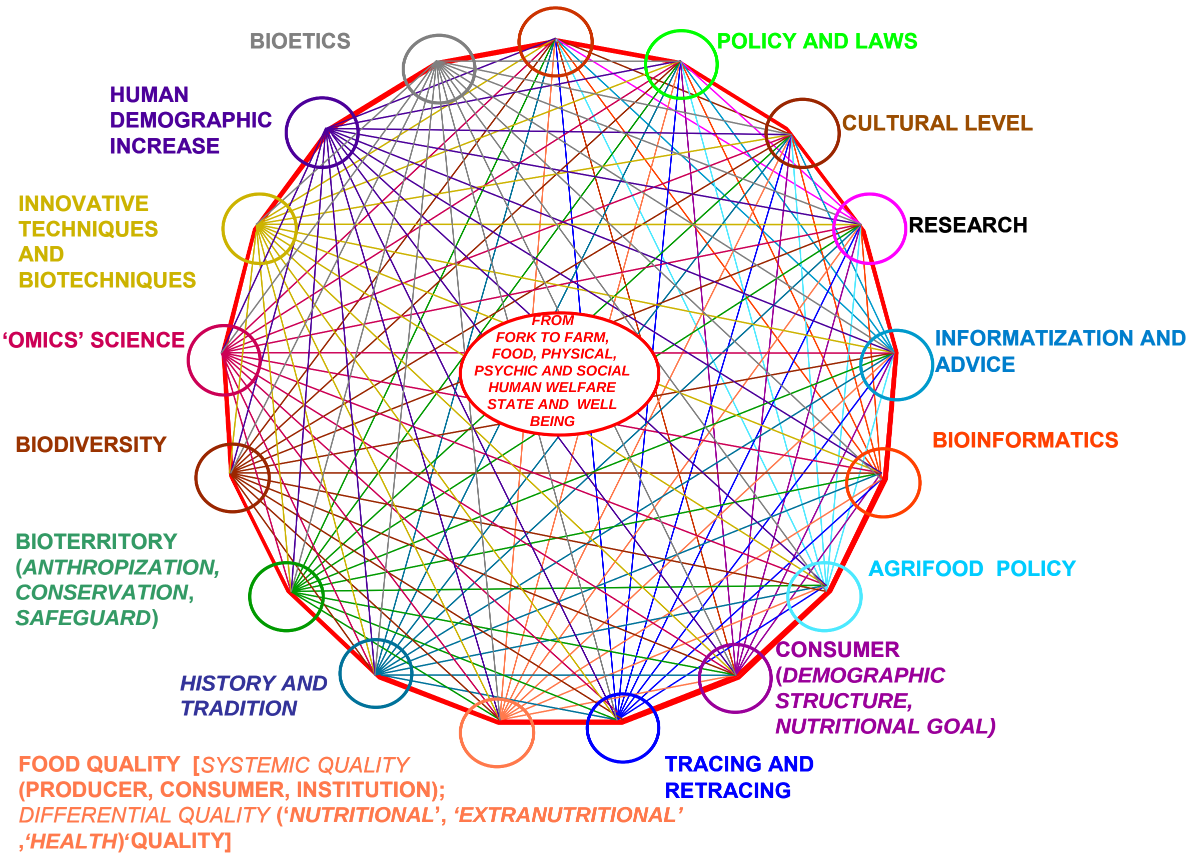
Biodiversity was defined by the European Agricultural Commission (DG AGRI,1999) as: *"…the variability of the life and its processes including all life forms, from the single cell to more complex organisms, to all processes, to pathways and cycles which link the living organisms to popolations, ecosystems and landscapes".*

Biodiversity is also an important element for '*biomimicry*'. This is a new scientific discipline whose objectives are the design and building of simple and/or complex systems typically taking inspiration from *naturality*. Some '*biomimicry*' sectors are: *biomedical engineering*, '*artificial*' *intelligence*, *nanotechnologies*, *robotics* (*electronic nose, biomimetic robot*), *bioarchitecture*, etc..

The safeguard of endangered animal genetic resource plays a fundamental role for the following reasons: (i) *biological*; (ii) *socio-economical*; (iii) *cultural*; (iv) *juridical*; and (v) *ethicalBiological reason*. An animal is not identifiable only with its reproductive and productive function, but it also bears important but yet poorly known biological-evolutive information useful for characterising its '*individuality',* especially in the light of complexity of its structural and functional genome.

The appearance of new phenotypes is *'unforeseen*' neither it is '*production from nothing* but it is always a transformation of previous '*potentialities*'. Thanks to this potentiality, organisms actively participate in the 'building' of the microenvironment in which they live. In 1907 H. Bergson in its book '*L'èvolution crèatrice*' proposed the term '*creative*' to mean *'èlan vital '*(vital rush) in the sense of *"capability to produce a continuous flow of evolutive novelty"*. This potentiality is deeply conditioned by the *'irreducible complexity'* (Matassino, 2007).

Figure 1. Exemplification of *'mandala'* representative of human physical, psychical and social welfare and well being in relation to *'food quality'* (*from fork to farm, food, well being*) (Matassino and Cappuccio, 1998).



J. Melise (cited by D. Matassino, 2001) pointed out that *"'complexity' is not that absolute harm that the French rationality searches out in the name of the clearness, homogeneity and universalism. On the contrary, it is the recognition of the richness of any size and nature of the living organization"*; really it is a true '*complex system*' (Matassino, 2001) since that: (i) it must be delimited in its borders time by time; (ii) it must be known in its *qualitative* and *quantitative* components and their relationships; (iii) it is spatially and temporally *'flexible'* and *'variable'* due to its unstable frameshift; (iv) it is characterised by differentiated *'constructivism capacity'* due to the information stored in the time and in the space; (v) it is deeply *'autoregulative'* and *'homeostatic'*, so that it may produce novel combinatory interactions among its components able to give rise to dynamic and peculiar *'statibus'* whose rules may spatially and temporally change similarly to '*genome system*' in which continuous novel combinations among DNA segments (also due to the transposons) and selective mutations occur; (vi) it is not a linear model in the sense of *absence* of *'cause –effect'* relationship; (vi) it is '*unpredictable*', in the sense that it isn't totally '*computable*' because it is characterised by '*deterministic chaos*'; (vii) it has a '*peculiarity*': it is a *'subjective universe'* not identifiable with a mere object of *reductionism*, but it is *discernible* and endowed with its own *'alterity'* ; and (viii) it has a specific pertinence: to put in 'dialog' the various components'.

The biological diversity is the only which allows to have at one's disposal *'genetic information'* able to favour, in the future, the *'constructivism capacity'* of living beings in occasion of unpredictable changes of both environmental conditions and needs of '*bioactive*' molecules with *'nutritional'*, *'extranutritional'* and '*health'* function for human beings.

As reported by Matassino *et al.* (2007), the '*irreducible complexity*' of the single cell has an '*unique*' role as it undergoes to continuous perturbations (information) solidifying into *intra* and *inter* cellular interactions, in addition to the interactions between genome (nuclear and mithocondrial) and various '*environmental*' factors (the so-called *'non genetic'* factors).

This '*irreducible complexity*' is always object of discussion, such as the opened debate on Weissman's somatic – germ' dualism (1883, 1885), which traces its origins back to the ending of 1800 and was then revived on experimental and theoretical basis by Waddington (1942, 1953, 1957) with the concepts of: (i) *'epigenetic landscape'*; (ii) '*canalization of development*'; and (iii) '*genetic assimilation*'.

The *'epigenetic landscape'* is identifiable with a series of *'development routes'*; each route arises from a step in which active DNA segments branch new routes; therefore, each development phase is, in turn, a real '*outrider*'for the subsequent step.

The '*canalization of development'* (Matassino *et al.*, 2007) includes all the behaviours of a living being leading to '*constructivism capacity*' which realises canalizing (constraining) the development to proceed to alternative routs as environmental conditions vary. According to English '*realistic*' philosophy referable to '*'évolution créaticé*' concept introduced by Bergson (1907), the '*evolution*' might not occur without '*canalization*' deeply dependent on one or more constraints. A '*constraint*' is "*a factor which constrains conditioning, both positively and negatively, the phenotypic changes towards a direction established by past or formal history, rather than the current adaptation"* (Gould, 1989). According to Bettini (1972), this '*canalization*' is allowed by '*dams*', identifiable with '*plans of cosmic organization'* which regulate its flow.

The *'genetic assimilation'* is defined as phenomenon consisting into a '*phenotypic change'* due to the environmental perturbations (identifiable with '*phenotypic plasticity*'), firstly not heritable, but then, able to become heritable from generation to generation; '*genetic assimilation*' is explained by evolutionists as manifestation of a '*cryptic*' genetic variability expressed in a given microenvironment thanks to a selective advantage. It is thought that environmental triggers may favour the expression of a '*cryptic genetic variability'* and the related phenomena, drawn by environment through natural selection '*screening*', may be genetically assimilated. This event is close by '*capacitation*' phenomenon, which is due to the environmental stress, whose effect solidifies into a reactivation of '*repressed genetic potential'* which express as appearance of new phenotypes.

Social-economical reason. AGTs and AAGTs, especially reared in '*marginal*' areas where intensive production model cannot be applied due to lack of economical presuppositions that make it convenient, are the only organisms able to play a zootechnical role, taking into account their own productive capability exploiting nearly exclusively local pasture (Matassino *et al.,* 1993).A '*local typified labeled product*' (LTLP) is a clear example where use of biodiversity, linked to a variegated endogenous resource of a '*bioterritory*', is a prime and irreplaceable element.

Not all inventoriable zootechnical diversity must be preserved; it is difficult to make precise and concrete choices in the preserving a given genetic type in danger of erosion; among the various theoretical strategies what that suggested by Weitzman (1992, 1993) concerning conservation policies is very current:*"the application of proper 'diversity fuctions'"*. The use of *'diversity function'* allows to make proper choices for *'short term'* and *'long term'* conservation politics. Metrick and Weitzman (1996) and Ollivier (1998) suggest an interesting procedure to decide the endangered genetic type allowed to be conserved on the basis of scientific arguments of *'rarity'* and *'uniqueness'*.

The aforesaid Authors evidence with mathematical statistical procedures the possibility to draw up a priorities list simultaneously concerning *'rare'* AGT and '*unique'* AGT concluding that *"in the priority classification a genetically 'rare' AGT may first come an 'unique' AGT"*. These procedures are particularly important in the deciding the AGT to be *'cryoconserved'*.

The undoubtly difficult choice is also an important action in order to individuate a proper balance between species to be safeguarded. These difficulties are partially overcome by a series of considerations about *'microbioterritory'* or *'microagroecosystem'* where the *'rare'* or *'unique'* animal is reared, based on: (i) cultural environment (tradition, etc.); (ii) social environment; and (iii) economical environment.

Cultural reason. AGTs and AAGTs can be considered like *cultural goods* because they form a patrimony with special value of historical and biological documentation; therefore, it is a duty of the community to transmit this patrimony to the future generations. The value of an AGT as '*keeper of local traditions in the rural areas*' has to be considered for its role in the: (i) landscape conservation, intended as percentage of farms which contribute to the future maintenance of traditional farm landscape related to AGT; (ii) maintenance of gastronomy, intended as relationship between the AGT and its products or typical dishes; (iii) maintenance of folklore, intended as collection of local traditions directly or indirectly linked to AGT; and (iv) maintenance of handicrafts, intended as putting in practice of local handicraft forms, directly or indirectly linked to AGT.

Juridical reason. The *ancient biodiversity* bears a particular patrimony whose juridical character derives not only from privatistic nature of the good but also, and perhaps still more, from the general nature of the interest in the social utility and conservation of the patrimony itself (Mazziotta and Gennaro, 2002).

It is foreseen a *'biological federalism'* able to *'confer again'* importance and dignity to the *'ancient autochthonous biodiversity'*; this *'biological federalism'* configures a *"new subject in the 'law' sector for the contextual presence of the elements responsible for the juridical relevance of good and which allow to recognize its juridical character"*.

Ethical reason. Biodiversity conservation must be regarded as '*ethical imperative'* since that it represents, not only a patrimony that must be defended and transmitted to the future generations to improve '*life quality'* but, also, a patrimony that has the right to its own existence for its intrinsic properties; biodiversity respect is oriented towards species in its globality; the 'individual' or the 'single subject' (intra- specific diversity) have not disregarded; species may be considered an abstractedness since that it doesn't suffer while the individual suffers and dies, leading to the end of 'species' and its '*genetic diversity'*; the '*single*' has fundamentally 'important' rights including not only the right to life but also that to conservation of genetic integrity; indeed, any reduction of genetic variability is a dangerous loss for the '*all*' (Matassino, 2002).

Zootechnical Book

In 1979 Matassino proposed the need for the implementation of' *zootechnical book*' for recording of reproductive and productive parameters of each AGT or AAGT for its better temporally and spatially use and valorization.

Law 30/91 stated that: *"Zootechnical Book is intended as book managed by a national breeders association with juridical personality or by body of public right, in which sire animals of a given breed and their ancestors are reported*". For breeds and populations at limited diffusion *'simplified herdbook'* are arranged. The aim of their institution is not to operate a selection on these populations, often in danger of extinction, but to preserve their genetic patrimony for its high historical and cultural value, also valorising their productive potential and implementing their use in peculiar environmental conditions.

In Italy, several zoological books, within each taxonomical group, exist: (i) zootechnical book for cattle autochthonous populations and ethnic groups at limited diffusion, established in 1885 and currently managed by Italian Farmers Association (AIA), with the aim to perform a periodical standardisation of birth register and to make easy the elaboration of data concerning safeguard activity (somatic evaluations, and, for the most endangered populations, setting up of reproduction plans to preserve genetic variability); for the most endangered populations a periodical *screening* of genetic variability is carried out in order to individuate the sires destined to *Instrumental Insemination* (II); (ii) zootechnical book for sheep and goat populations at limited diffusion, established in 1997 and currently managed by National Association for sheep-rearing (Asso.na.pa), aimed at making a periodical standardisation of birth register and monitoring the registered population size ; (iii) zootechnical book for pig populations established in 2001 and currently managed by National Pig Farmers Association (ANAS); it registers genealogical information of the registered subjects in order to preserve populations with particular reference to the maintenance of their genetic variability and valorisation of their economic potential; and (iv) zootechnical book for Equids populations classified as local ethnic groups, established in 2003 and currently managed by AIA; database management includes a supervision of recording activity, carried out by Provincial Farmers associations and horse Improvement institutes through a specific software for information management; currently, conservation activity consists of registering population size of each AGT.

CONCLUSIONS

It is foreseen a *'biological federalism'* able to *'confer again'* importance and dignity to *'ancient autochthonous biodiversity';* this *'biological federalism'* configures a *'new subject'* in the '*law'* sector.

A *NFP*, in the frameshift of GS.FAO.MANGR represents an '*institution*' of reference with variegated functions aimed at safeguarding of genetic resource in danger of extinction and/or abandonment; this safeguard must be rational and shared by institutions and interested operators.

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Ruolo del Registro anagrafico per la conservazione dei tipi genetici autoctoni

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ANAS - Associazione Nazionale Allevatori Suini (RM)

SUMMARY - The Register was instituted by Law 30/91 and it has been running since 2001. The goal of Register is the conservation of Italian native breeds and their economic valorisation through pedigree information collection and mating scheme managing aimed to preserve genetic variability and to keep average inbreeding low. Currently there are six recognized native breeds, and interesting plans are under way to exploit local market niches.

Key words: Register, native breeds, conservation

INTRODUZIONE

Agli inizi del '900 si contavano in Italia 11 popolazioni di suini autoctoni con una discreta consistenza: Cinta Senese, Cappuccia (o Chianina), Maremmana (o Macchiaiola, o Romana), Mora Romagnola, Perugina, Abruzzese, Casertana, Pugliese, Calabrese, Siciliana e Sarda (Mascheroni, 1927). La situazione andò progressivamente deteriorandosi nel corso del secolo XX e negli anni '80 la Cappuccia, la Maremmana, la Perugina, l'Abruzzese e la Pugliese risultavano definitivamente estinte, mentre delle altre si registrava qualche sparuta presenza o solamente tracce in soggetti meticci.

Nei medesimi anni '80 si assiste tuttavia ad un risveglio dell'interesse sia da parte delle Amministrazioni pubbliche, tra cui la Commissione UE, sia da parte della comunità scientifica verso i problemi della conservazione della biodiversità animale e vegetale. Risale al 1986 il programma della Unione Europea per la costituzione di una rete di banche delle risorse genetiche suine "*European gene banking project for pig genetic resources* - GENRES 012". In Italia l'iniziativa, che consisteva nello stoccaggio di materiale seminale congelato, riguardò la Cinta senese, il Nero siciliano e la Mora romagnola. Tra le iniziative adottate in quegli anni, va ricordato il sostegno finanziario della Regione Toscana ad alcuni allevatori interessati all'allevamento della Cinta senese, intervento che ha permesso di censire nel 1986 una popolazione di 81 scrofe e 3 verri di quella razza. Cogliendo i primi segni di un rinnovato interesse per la Cinta senese, nel 1996 il Libro genealogico della specie suina istituì una sezione apposita per quella razza avviando la prima esperienza di gestione centralizzata di una razza suina autoctona. Successivamente, la Legge 3 agosto 1999, n.280, affidò il Registro anagrafico della specie suina, già istituito dalla Legge 15 gennaio 1991, n.30, all'Associazione Allevatori che già deteneva il Libro genealogico di specie, cioè all'ANAS. Il Disciplinare e le Norme tecniche del Registro anagrafico furono approvati con DM 20871 del 6.3.2001, e poco dopo la Cinta senese venne trasferita dal Libro al Registro anagrafico.

Finalità del registro anagrafico

Il Registro "*conserva le informazioni genealogiche dei soggetti iscritti al fine della conservazione delle razze autoctone con particolare attenzione al mantenimento della loro variabilità genetica e promuovendone al contempo la valorizzazione economica*." (art 3 Disciplinare del L.g. e del R.a. della specie suina DM 6485 del 30.05.2007). Il Registro dei tipi genetici autoctoni attualmente riconosce sei tipi genetici: la Cinta Senese, la Mora Romagnola, la Calabrese, la Casertana, il Nero Siciliano e la Sarda.

E' utile ricordare che, ai sensi dell'allegato alla Legge n. 280/99, la differenza tra un Libro genealogico ed un Registro anagrafico è che quest'ultimo non prevede alcun programma od intento selettivo. Da ciò deriva che i tipi genetici autoctoni sono interessati solo a programmi di mera conservazione ai fini del mantenimento della loro (residua) variabilità genetica. Ogni eventuale attività di miglioramento genetico comporterebbe di contro una riduzione della variabilità genetica, aspetto già critico per queste razze, e addirittura potrebbe rendere meno interessante la conservazione del tipo genetico in questione attenuando la sua differenziazione dalle razze cosmopolite.

Il Disciplinare del Libro genealogico e del Registro anagrafico è molto preciso in proposito: l'art 21 ribadisce infatti *"la conservazione o salvaguardia dei suini delle suddette razze si persegue mediante il consolidamento delle caratteristiche di rusticità anche attraverso il contenimento della consanguineità, nonché il miglioramento delle prestazioni produttive e riproduttive in condizioni di allevamento brado o semibrado mediante l'aumento della capacità di sfruttamento delle risorse alimentari disponibili nelle aree marginali"*

Dato che la variabilità genetica da conservare è quella che si trova nella popolazione tradizionale, una volta raggiunta una ragionevole certezza di aver identificato tutti i capi sopravvissuti di una determinata razza, il Registro anagrafico provvede a "chiudere" le registrazioni rifiutando l'iscrizione di suini di ascendenza sconosciuta. Tale "chiusura" è già stata adottata per le razze Cinta senese e Mora romagnola.

Organizzazione registro anagrafico

L'indirizzo dell'attività del Registro è stabilito dalla Commissione Tecnica Centrale, composta da esperti nominati dal Ministero delle Politiche Agricole Alimentari e Forestali, da rappresentanti del medesimo Ministero e di quello della Salute, da rappresentanti delle Regioni, degli allevatori e da un rappresentante dell'ANAS..

L'Ufficio centrale presso l'ANAS ha il compito di coordinare ed attuare l'attività prevista dai Disciplinari e dalle delibere della Commissione Tecnica Centrale. Gli Uffici periferici presso le Associazioni allevatori locali sono incaricati della raccolta dei dati negli allevamenti e dell'identificazione dei soggetti.

E' previsto un flusso dati quindicinale dagli Uffici periferici verso l'Ufficio centrale del registro anagrafico. L'operatività è dettagliatamente regolamentata dall'insieme delle norme del Disciplinare, delle Norme Tecniche e delle Norme Operative (documenti liberamente consultabili sul sito web anas.it). L'attività di Uffici periferici ed allevamenti iscritti è sottoposta alla verifica dell'Ufficio centrale, che vi provvede sia attraverso l'analisi dei dati ricevuti sia mediante visite ispettive.

Il Disciplinare stabilisce, tra l'altro, le caratteristiche di ciascun tipo genetico, nonché le cause di esclusione che ne prevengono l'iscrizione al Registro anagrafico. In particolare, con DM 24089 del 18 dicembre 2006 sono state apportate importanti modifiche alla descrizione delle razze autoctone. L'iniziativa è stata deliberata dalla Commissione tecnica centrale che, sulla base della proficua esperienza maturata dopo i primi anni di attività del Registro e tenendo conto della documentazione riguardante le popolazioni originarie, ha ritenuto opportuno precisare meglio i caratteri distintivi di ogni tipo genetico. Questo aggiornamento ha ulteriormente qualificato l'attività del Registro, permettendo una più marcata caratterizzazione delle sei popolazioni interessate.

E' utile far presente che nel caso della Mora romagnola, accogliendo la richiesta degli allevatori, l'Ufficio centrale ha provveduto direttamente alla verifica dei soggetti presenti in tutti gli allevamenti iscritti. Sono così stati esaminati 357 capi, dei quali ben il 34 % presentava caratteri di esclusione.

Situazione tipi genetici autoctoni

Nella tabelle di seguito sono riportati i dati 2006 della consistenza e delle prestazioni riproduttive distinti per tipo genetico.

Tipo genetico	Verri N.	Scrofe N.	Allievi Maschi N.	Allievi Femmine N.	Nuclei N.
Cinta senese	192	927	514	969	156
Nero siciliano	30	173	297	347	22
Mora romagnola	54	144	428	380	38
Calabrese	20	72	122	150	13
Casertana	22	68	136	195	10
totali	318	1.384	1.497	2.041	239

Cinta Senese	n. Parti	media	Dev. Std.	Min	Max
N. nati tot	1137	6,91	2,14	1	14
N. nati vivi	1128	6,34	2,19	1	13
N. svezzati	1030	6,04	2,14	1	13
Età al parto (gg)	1137	1115,22	527,76	265	3714

Nero Siciliano	n. Parti	media	Dev. Std.	Min	Max
N. nati tot	180	6,55	1,72	1	12
N. nati vivi	180	6,23	1,71	1	11
N. svezzati	110	6,15	1,90	1	11
Età al parto (gg)	180	1360,26	592,35	407	2754

Mora Romagnola	n. Parti	media	Dev. Std.	Min	Max
N. nati tot	177	7,10	2,60	1	14
N. nati vivi	175	6,89	2,49	1	13
N. svezzati	163	5,46	2,38	1	13
Età al parto (gg)	177	657,65	289,47	2881	1960

Calabrese	n. Parti	media	Dev. Std.	Min	Max
N. nati tot	83	6,60	2,78	1	13
N. nati vivi	80	6,13	2,66	1	12
N. svezzati	59	5,32	2,14	1	10
Età al parto (gg)	83	952,88	491,05	265	2547

Casertana	n. Parti	media	Dev. Std.	Min	Max
N. nati tot	91	7,36	3,11	1	14
N. nati vivi	91	7,07	3,03	1	14
N. svezzati	66	4,95	2,65	1	11
Età al parto (gg)	91	762,08	329,23	296	1679

I dati di consistenza confermano che la razza Cinta senese, la prima ad essere interessata dall'attività di conservazione (1996), ha raggiunto una discreta consistenza di riproduttori (verri e scrofe) e di allevamenti.

Per quanto riguarda le altre razze, Mora romagnola e Nero siciliano registrano un graduale consolidamento, mentre più esigua è la consistenza di scrofe ed allevamenti.di Casertana e Calabrese. In ogni caso è doveroso segnalare l'inversione di tendenza, registrata nel corso del 2006, che ha avvicinato e sta avvicinando all'attività di salvaguardia di questi due tipi genetici alcune nuove promettenti realtà di allevamento. Per la razza Sarda, ultima riconosciuta con DM 8 giugno 2006, l'operatività del Registro è iniziata nel corso del 2007 con l'iscrizione dei primi 7 allevamenti.

Le popolazioni attualmente allevate derivano da uno sparuto gruppo di soggetti (qualche verro e scrofa) "fondatori". Pertanto esse sono caratterizzate da elevati coefficienti medi di parentela e di consanguineità. Questa situazione richiede una attenta gestione della riproduzione. In ogni caso va rilevato che la struttura demografica delle razze autoctone italiane, con poche scrofe allevate per nucleo - dalle 3,8 della Mora romagnola alle 7,9 del Nero siciliano - rappresenta in realtà un vantaggio ai fini della loro conservazione e del mantenimento della loro variabilità genetica. Anche a causa di questa distribuzione frammentaria, il rapporto tra verri e scrofe è particolarmente elevato, con 1 verro ogni 4,4 scrofe. Meglio di altre, questa condizione garantisce il mantenimento della variabilità genetica originale.

I servizi forniti dal Registro incoraggiano e sostengono questa organizzazione produttiva, che del resto ricalca i modelli aziendali arcaici nei quali le razze autoctone venivano allevate con successo.

Per quanto riguarda le prestazioni riproduttive si rileva che non esistono differenze apprezzabili tra i diversi tipi genetici, ed esse rimangono sempre significativamente inferiori a quelle delle razze migliorate.
Interessante il dato dell'età media al parto, che almeno nelle razze Cinta senese, Nero siciliano e Calabrese evidenzia un intervallo di generazione particolarmente lungo ed una buona longevità delle scrofe. Anche questo parametro demografico aiuta a contenere l'aumento di consanguineità.

Gestione della riproduzione

In relazione a quanto sopra illustrato, il Registro anagrafico ha adottato alcuni accorgimenti per la gestione della riproduzione finalizzata al mantenimento della variabilità genetica residua.
Gli aspetti più rilevanti di questa gestione sono i seguenti:

a. *Numerosità maschi*.
   Ciascun nucleo iscritto, per quanto costituito da poche scrofe, deve tenere almeno un verro, anche se ciò comporta un profilo di minore efficienza economica dell'allevamento. In particolare si segnala che la realtà della Mora romagnola si distingue per il più alto rapporto tra verri e scrofe (1 ogni 2,7), seguita dalla Casertana (1 ogni 3,1), dalla Calabrese (1 ogni 3,6), dalla Cinta senese (1 ogni 4,8) e dal Nero Siciliano (1 ogni 5,8).

b. *Scelta entro famiglia*.
   La scelta della rimonta dei maschi e delle femmine deve essere effettuata da tutte le famiglie presenti. I dati della consistenza degli allievi maschi e femmine iscritti nel 2006 dimostrano la positiva attenzione riservata dagli allevatori a questo aspetto.
c. *Ricambio verri*.

Data la ridotta dimensione degli allevamenti, i verri necessitano di un adeguato ricambio (consigliato ogni 18 mesi) per escludere un loro improvvido utilizzo sulle proprie figlie.

d. *Controllo della Riproduzione e Consanguineità*.
Gli allevamenti devono possedere strutture per realizzare i gruppi di monta e confinare le scrofe allattanti al fine di garantire la corretta attribuzione dell'ascendenza a ciascun suinetto. E' liberamente disponibile sul sito www.anas.it ogni informazione genealogica e soprattutto il calcolo *on line* del coefficiente di consanguineità di ogni soggetto iscritto e di ogni ipotetico accoppiamento. Ciò consente agli allevatori una più precisa pianificazione della riproduzione. L'utilizzo di questi servizi informativi da parte degli allevatori iscritti è una pre-condizione per una efficiente ed efficace azione di salvaguardia della razza.

Valorizzazione produzioni

La valorizzazione economica delle produzioni è di primaria importanza per mantenere in efficienza programmi di conservazione *in situ.*.

La polverizzazione produttiva (allevamenti e piccoli laboratori di lavorazione delle carni) ed i ridotti volumi produttivi costituiscono seri limiti per la gestione di una offerta rivolta ai canali distributivi moderni. Inoltre, la fragilità economica del tessuto produttivo fa sì che scarseggino risorse per la promozione dei prodotti, nonché per eventuali interventi volti a migliorare la preparazione e la presentazione dei prodotti stessi. Quanto sopra comunque non impedisce lo sviluppo di metodi di commercializzazione alternativi ed innovativi comunque in sintonia con le regole del mercato. Per esempio, lo sfruttamento delle sinergie con i servizi del settore turistico ed agrituristico, l'inserimento nella distribuzione dei prodotti dell'artigianato locale, la presenza nella ristorazione sia tipica che innovativa, l'abbinamento a denominazioni già affermate (vinicole, dei formaggi, ecc..), la vendita telematica, le produzioni biologiche. In ogni caso sembra necessario che l'allevatore eserciti direttamente o si associ ad attività di lavorazione delle carni per poter vendere i prodotti trasformati.
Le politiche di valorizzazione possono essere rafforzate dalla creazione di marchi di qualità, che possono essere privati o pubblici (DOP, IGP). Anche quando ne ricorrano le condizioni, la scelta tra la registrazione di un marchio privato o la richiesta di riconoscimento di un marchio europeo (DOP, IGP) deve essere accuratamente valutata sotto il profilo dei costi-benefici. Il rischio, quando si opta per un riconoscimento europeo, è quello di istituire una filiera di certificazione con costi di funzionamento (organismo di controllo indipendente, ecc.) che potrebbero non trovare adeguata compensazione vista l'esiguità della base produttiva. In ogni caso, la possibilità di utilizzare Istituti di controllo consortili partecipati da tutta la filiera suinicola ed operanti senza fini di lucro consente comunque di limitare i costi di controllo al minimo necessario.

Per certe produzioni, un grosso limite potrebbe derivare dall'impossibilità di raggiungere una massa critica di produzione necessaria per sostenere la commercializzazione del prodotto. In tal senso, molto si è discusso circa l'opportunità di prevedere un sistema produttivo che impieghi anche meticci con altre razze.

La situazione di rischio d'estinzione delle singole razze ha suggerito finora di dedicare tutte le energie alla riproduzione in purezza, ma oggi la gestione dei programmi di conservazione è in via di consolidamento, e non si può escludere che in un futuro più o meno prossimo si verifichino le condizioni per valutare l'organizzazione di sistemi produttivi che comprendano, ben distinte, sia la produzione in purezza che in incrocio. Anche l'allevamento degli incroci fa parte della tradizione: il così detto suino *grigio o tramacchiato* è stato per decenni il prodotto più diffuso in Toscana, ottenuto accoppiando la razza Cinta Senese con la razza Large White, mentre il *fumato* era ottenuto dall'incrocio tra Large White e Mora Romagnola. Ancora, le caratteristiche dell'incrocio tra Large White e Casertana furono scientificamente documentate dal prof Baldassarre già nel 1899, ed è interessante ricordare come già in quel lontano lavoro si individuasse con chiarezza nell'efficace mantenimento della razza pura la chiave per il successo della produzione in incrocio.

In ogni caso la scelta del sistema produttivo più adatto, assicurata la "messa in sicurezza" della razza, non può prescindere da una attenta valutazione economica ed organizzativa del mercato di riferimento per i prodotti derivati dal tipo genetico autoctono (in purezza o in incrocio) e dei possibili scenari futuri.

Considerazioni conclusive

Le razze autoctone italiane rappresentano una realtà importante sotto il profilo storico, culturale, sociale ed economico. La loro salvaguardia è una delle componenti qualificanti ed imprescindibili delle politiche per lo sviluppo di modelli produttivi sostenibili, fortemente integrati con attività di tipo "agrituristico". L'interesse del mercato per i prodotti derivati da questi sistemi produttivi assicura prospettive di sviluppo promettenti e può garantire nel prossimo futuro le necessarie risorse per il consolidamento della conservazione di queste razze.

L'attività del Registro anagrafico ha dimostrato la sua importanza ed efficacia per la salvaguardia di questi particolari "tipi genetici". I servizi informativi, quali sono quelli disponibili sul sito *www.anas.it* alla voce Registro anagrafico, ed il rigoroso rispetto delle norme che regolano l'utilizzo dei riproduttori hanno dimostrato la loro efficacia per una gestione delle razze coerente con gli obiettivi della conservazione.

La disponibilità di una popolazione di soggetti puri con una sufficiente variabilità genetica e con caratteristiche di razza ben definite rappresenta il punto di arrivo del lavoro del Registro anagrafico. Tale disponibilità è però il punto di partenza per i programmi di utilizzo di questi tipi genetici che, anche eventualmente in incrocio, possono produrre carni per un mercato di nicchia di prodotti molto differenziati.

Nero Siciliano Pig

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SUMMARY – The presence of a black pig in Sicily, characterised by high rusticity, quasi wild, reared in complete freedom in the woods, it is mentioned from Omero. Its origin dates back to Greek-Carthaginian period (8^{th} - 7^{th} century B.C.) and even in Rome, the production of a Sicilian pig was well known as early as the 2^{nd} century B.C.. Nero Siciliano pig is also present in the Middle Ages until now, showing a slight reduction during the Arabian period. Some Authors indicated that Nero Siciliano pig could come from an autochthonous breed, for Others Neapolitan subjects have contributed; Montanaro (1939) indicated that Iberian blood (Sanson) was introduced in the Nero Siciliano pig. Chicoli (1870) described the presence in the Island of several populations, called with the names of some Sicilian districts. The blood of many breeds was introduced in Nero Siciliano pig, such as the "Napoletana – Casertana" breeds which were used in the constitutions of some nucleus present in Calascibetta, Nicosia (Enna) and Mistretta districts (Messina). The most significant presence of Nero Siciliano pig is in the Madonie area, mainly in the Nebrodi mountains (Messina), where it is called "Nero dei Nebrodi" pig ecotype of Nero Siciliano pig, nowadays object of attention. The living system as well as the continuous search of food have determined some problems for the environment, including inhabited areas, with consequent ostracism and damages towards the biodiversity. Understanding that the extensive management do not represent a biological rearing system and the observation of haematic parameters, index of metabolic-nutritional *status*, not very encouraging, have suggested basic conditions of well-being for the black pigs living in the woods; the animals together with the undergrowth products (mainly acorns) received integrations with traditional feed: a type of "élevage en plein air". Positive results for the environmental protection and for the quantitative and qualitative characteristics of the productions, studied by physical and chemical analyses and specific panel tests, were obtained. High fat deposition (lard) for the pigs reared in extensive and plein air conditions, with interesting characteristics of fatty acid profile for human health were observed. Moreover, polymorphism of some candidate genes for productions performance and phenotypic traits, such as meat quality, growth, carcass adiposity, coat colour, reproductive performances, etc. represent important information for selection processes and traceability.

Key words: Nero Siciliano pig, origins, rearing systems, productions.

INTRODUCTION

The Nero Siciliano pig has been, especially in the past, the expression of ethnic-genetic heterogeneity with clear polymorphism more or less accentuated in relation to the areas and breeding systems and obviously, to the type of programmed production. Thus, in the internal areas and/or more or less impassable, less sensitive to external conditionings, the animals have conserved, in general, the characteristics of the ancient pig, rather close to the wild boar. The variants, usually individuated in general terms, according to Pino (1947), are to be referred to the Casertana (Pelatella), and later to the Cinta senese, to the Parmigiana among the Italians, to the Large Black, to the Large White, to the Berkshire among the English, and to the Chester White and to the Poland China among the Americans (Pino, 1947).

The Casertana appears among the most ancient introductions into Sicily, even if discontinuous for motives which are often more commercial than zootechnical, and this also regards the so-called Napoletani, in general from Puglia, (Pino, 1947) and it is the breed that was to leave the greatest signs in the Nero Siciliano pig, to be encountered in a more evident manner (Chiofalo, 2000c) in the black Calascibetta pig and in those of other Sicilian areas; and also Marchi (1897) who refers to the influence of the Napoletana and for Cassella (1921) who speaks about the pig of Calascibetta (province of Enna). The latter adds that the Nero Siciliano pig, as the North African one, perhaps comes from the Napoletano which underwent a considerable change in Sicily on account of the lack of pastures and in certain areas also of woods.

Even the Experimental Zootechnical Institute of Sicily, besides Large Black and Large White reproducers, imported the Casertana subjects characterised by low fertility and affinity with the Nero Siciliano pig. The Large Black directly introduced into Sicily from England has also been present in Sicily. As it is an excellent grazer, characterised by very large, long and drooping ears, this would limit its visibility and therefore the possibility of running about in the pastures in favour of the intensive use of same, also with the only advantage, thanks to its black coat, of facing the solar radiation better: an attitude for lard and meat.

The Large White, introduced into Italy by Zanelli and later into Sicily by Tucci, Director of the Experimental Zootechnical Institute for pure and/or cross breeding has had almost no results for breeding in an extensive condition, contrary to the industrial or localised breeding. The absence of rusticity, the white coat and the depigmentation, rather negative for sunrays, the receptivity of diseases, and the feeding exigencies were unfavourable for it. The Berkshire, a breed for lard, with a fleeting apparition in Sicily, was the initiative of cross-breeding with local pigs (Pino, 1947).

The presence of this black pig in Sicily, rustic, almost wild with a congenial habitat in the woods, also judging from the fossil remains and from the numerous bibliographical references was ascertained in very olden times before the 8^{th} – 7^{th} century B.C. This animal, with its system of life, generally reared in extensive conditions is to be found throughout Medieval Times, except for some decrease during the Arab domination for well-known religious motives, arrives to the present day.

Generally, the Nero Siciliano pig is set as an “Indigenous Sicilian ” population which for some experts (Tucci, 1908; Giuliani, 1940) would derive from a local autochthonous line; for others, (Faelli, 1928) subjects of the “Napoletana” and for (Montanaro, 1939) the Nero Siciliano pig would belong to the “Iberian type of Sanson”.

Different swine breeds/populations were listed by Chicoli (1870) in Sicily, all leading to the “Napoletana”, according to this Author, and with an “ordinary” black coat: a *breed of S. Agata di Militello* present in all the province of Messina and in part in the provinces of Catania and Syracuse; a *Castelbuono breed* from the cross-breeding with that of the Cape of Good Hope; the *Trapani breed* present in the province of Trapani, Agrigento and Caltanissetta, more rustic than the previous ones; the *Patornese breed* present in the Etna area, near the Sus Scrofa; the *Cesarotana breed* (province of Messina) and the *Troina breed* in the province of Enna. In 1929 Romolotti also affirmed that in the past, the existence of various autochthonous pig breeds was considered (of Calascibetta, Trapani, Comiso, Paternò, etc.) the origins of which were to be looked for in the cross-breeding with the Casertana breed, even though, on the basis of his study, he thought that the breed of the Nero Siciliano pig was the only one with well-delineated characteristics; and this could find a certain confirmation also in the one which Marchi and Pucci (1923) refer to, for which the Mediterranean areas would resent introducing foreign blood; for this reason, the better defined breeds would be found here. Besides, the system of life in denser woods and in the wildest areas would form a barrier to possible genetic pollution.

Present State

The Sicilian pig-breeding, as has already been mentioned, in its generality, has not a univocal physiognomy: as regards the same Nero Siciliano pig, Pino (1947) even speaks of the variety of the colour of the coat and of the coarse hair present only in 40%, and of adaptations and conformations connected to the possibilities of the different areas. For example, for this, according to the connection between woods and pigs, we find the greater concentrations of the Nero Siciliano pig, on the Madonie and above all along the mountain chain of the Nebrodi (the highest peak being 1847 metres a.s.l.) and of the Peloritani. The mountain chain of the Nebrodi – for the Arabs most of the territory was called “land of the woods” - with the Madonie to the South and the Peloritani to the East, can be considered the continuation of the Apennine chain in Sicily. With the continuation of rises and valleys it forms a landscape of great interest for its climate, characterised by considerable excursions during the year, the snow is also present for long periods, and for considerable varieties of vegetation that also regards pastures, where, besides the essences of pasture of a certain interest, uncultivated produce is to be found that has given life and sustenance to a consistent zootechnical patrimony (bovine, ovine, caprine, swine and equine), made up of autochthonous genetic types (TGA) of great meaning as regards bio-diversities and of a considerable productive potential. This is an interesting aspect, also for the action of mitigation on the climate, the presence of a woody area equal to 70,149 hectares distributed along the Nebrodi and Peloritani mountains. On the Nebrodi in seventy five per cent (75%) of the woods there are: Adriatic oak (*Quercus Cerris L.*), Evergreen oak (*Quercus ilex*), Cork oak (*Quercus Subera L.*), English oak (*Quercus sessilis Eh. Rh*) Beech (*Fagus selvatico L.*), Chestnut (*Castanea sativa Milla*), Maple (*Acer campestre L.*). Ninety five per cent (95%) is exclusively made up of oak and beech (Miceli, 1984). The presence of acorn of great interest is considerable, as we see, for the feeding of pigs, except those produced from the beech known for its negative aspects on fat.

Since 1993, the natural Park of the Nebrodi has existed which covers a surface of 85,587 hectares and is part of 21 communes of the province of Messina, Catania and Enna. In these territories, above all on the Nebrodi, the most significant presence of the Nero Siciliano pig is to be found which, also thanks to the orographic situation and in spite of, at times, the intervention of man, it has succeeded in conserving the most significant features such as uniformity, so much so that now we more frequently speak about the black pig of the Nebrodi, considered the most represented ecotype, and today it is well known; the studies carried out on this animal by us (Chiofalo *et al.,* 1989) presented at the International Congress of Ajaccio (Corsica) have also served to raise a whole series of attention towards the Nero Siciliano pig. It is a pig which has had its official recognition with the inscription at the Registry office (2001). The coat is black even if subjects with white faces and waists are present, very thick black slate skin on which coarse black hairs are implanted which, on the cervical-dorsal-loin line, with a dorsal stripe. The height at the withers is 60-65 cm. The forehead-nasal profile has a tendency to be straight, at times with signs of celoidism, instead, the direction and the carriage of the ears are less variable (Pino, 1947). It is an animal resistant to diseases and with a great power of adaptation in difficult environments; it has a considerable attitude to grazing and to procuring food. It has a discrete fertility, 6-7 piglets per litter, in relation to the conditions of breeding (prolificity: 7.6; mortality: 1.4%; weaned/born: 91.6%). At the age of one year, in the usual extensive conditions (wild), it reaches 60-70 kg. in weight. There is a considerable amount of lard on the neck, withers and back that appears to have a genetic characteristic. The present consistence in Sicily is as follows:

Estimated total consistency: More than 100 breedings; About 3000 subjects; About 1400 sows.
Data from native breeds Register ANAS: Breeding: 33; Subjects: 1534; Sows: 337.

A historical excursus, punctually dated, of the various racial introductions, on the Nero Siciliano pig, that trusts its origins to pre-history, and not all of them are mentioned, for obvious reasons of time, they say little about its formation, also for lack of scientifically correct data.

Recently, some researchers of the Departments MOBIFIPA of the University of Messina and of the DIPROVAL of the University of Bologna (D’Alessandro *et al*., 2006) have carried out some studies on the *MC1R* gene (Melanocortin receptor 1) in the Nero Siciliano pig, analysing the variability on a consistent sample of the animal in the optics of a possible utilisation of this *locus* for the traceability of the breed and of the relative productions, for a reliable authentication and above all as a contribution to the genetic characterisation of the Nero Siciliano pig. Taking into consideration (Russo *et al*., 2004) that the mutations of the *MC1R* gene act on the colour of the coat of the pig species being considered, some of them breed-specific (D’Alessandro *et al.,* 2006) and, therefore, of a considerable perspective for the procedures of recognition. The four allelic forms indicated for the *MC1R locus* are interesting. The most frequent allele was also observed in the Hampshire breed and some types of waist, observed in the black pig of the Nebrodi, Sicilian ecotype, bring us back to the former. An allele present in a smaller measure is found in the Duroc. There are also frequencies of alleles typical of the Large-Black, Pietrain, and Large-White – whose presence are ascertained in Sicily – as well as of the Meishan and the Landrace.

These studies, though with due reservation, affirm an appreciable genetic variability of the Nero Siciliano pig, an interesting premise for processes of target selection, and would confirm, in a scientifically correct way, some indications, already referred to, about the various introductions of blood in the Nero Siciliano pig, of others (Hampshire), already recalled by us in the past, and still of others (Landrace, Pietrain) recently utilised by single breeders in the perspective of more immediate results, not taking into consideration possible genetic pollution, even with a set of negative characters, as will be seen later. The alleles responsible for the black colour of the coat are more frequent, even if the intervention of other genes on the presence of the white parts of the body of this animal, admitted in the standard of the breed, is not to be excluded. These studies are important for the contribution of documentation on the history of the Nero Siciliano pig.

The research in this direction , operating on the Nero Siciliano pig (Russo *et al*., 2000) with the auxiliary of adequate instruments, has also faced in the DNA, the variability of some codifying genes for enzymes and proteins functionally known and connected with important characteristics of zootechnical interest, verifying polymorphism for all the loci analysed, some in non-codifying and others in codifying regions.

Some of these genes such as the sarcolipin (SLN), on the basis of their function and map position can be considered candidate genes for the growth and the adiposity of the carcass (Russo *et al*., 2003), as well as in the ATP1A2 *locus* (Russo *et al*. 2004) where there is the allele which is associated with the greater weight of lean cuts. The CSTB gene (cystatin B) is also found which, according to some (Andersson *et al*. 1994), seems to be significantly connected to the average weight gain. The CTSB *locus* (cathepsin B) has permitted to point out in the Nero Siciliano pig, allelic frequencies similar to those of the Pietrain breed, an ascertained presence in the history of the Nero Siciliano pig, with the prevalence of the allele 2 for which an association with the thickness of the back fat has been hypothesised (Russo *et al*., 2000).

Two alleles of the ESR *locus* (estrogen receptor) have been ascertained in the Nero Siciliano pig; one of these variants has also been pointed out in the Meishan breed, put in relation with the greater number of piglets born alive (Rothschild *et al*., 1996); its presence in the black pig is to be considered important, given the low prolificity registered for this animal, also for some perspectives of improvement of the reproductive attitude.

In the CRC *locus* (halothane sensitivity) of the black pig the presence of some subjects “Nn” has been ascertained, beside the prevalence of the allele “N”. Autosomal allele “n” at incomplete penetration, denominated gene for the sensitivity to the halothane associated with malignant hyperthermia (MH) and with the defect of the meat called as PSE. The allele “n” that could have been introduced in the Nero Siciliano pig through the cross-breeding with other breeds, among which the Pietrain, has already been documented. The individuation of the modification in the DNA has permitted to set up a test for the recognition of the carrier pig also from a single coarse hair (Russo *et al*. 1994).

The variability observed in the loci analysed, connected with productive and reproductive characteristics, outlines an interesting platform on the way of improving this animal with the possibility of selecting the interesting alleles and of increasing the frequency of them.

Productions

The Sicilian production of pig-breeding was known in Rome even before the coming of Christ (Chiofalo, 2000c; Chiofalo and Liotta, 2003).

The oldest Italian pork sausage would be the “salami of S. Angelo di Brolo” (province of Messina, Italy), produced with the meat of black pigs in the Mediterranean area (Barberis, 1997). It is an affirmation that should not surprise considering, writes Ballarini (2002), that, most probably, the first representation of salami is the one “found in Thebes in the tomb of the Egyptian Pharaoh, Ramsete III of the twenty first dynasty (1166 B.C.)” .

The fame of this Sicilian product, which outlives time, is to lead to the type of preparation and to the particular environment and ecosystem of the territory, to be compared to a great natural seasoning cell.

It is also to be remembered that the nutritional qualities of the meats of the Nero Siciliano pig have always been found excellent, both from a health and an organoleptic, almost hedonistic point of view, and the voluptuary aspects are often a testimony of genuineness and therefore of quality in the sense of bio-nutritional response. The lipid fraction of the meat, analysed by using ultra fast gas-chromatography has seen as the most important components oleic acid ($C_{18:1n9}$) equal to 43.8 – 45.7%, followed by palmitic acid ($C_{16:0}$) 21.7 – 23.9% and by stearic acid ($C_{18:0}$) 11.4 – 13.7%.

A considerable quantity of linoleic acid ($C_{18:2n6}$) 8.46 – 9.98% has been observed (Mondello *et al.* 2003). The organoleptic characteristics of S. Angelo salami prepared with the meat of the Nero Siciliano pig have pointed out (Liotta *et al*., 2003) a meat-fat ratio, tenderness, colour of the meat, grain and structure of the slices: ideal. The aromatic flavour and the colour of the fat are satisfying, the juiciness good. The chemical composition of the cured ham of the Nero Siciliano pig (Chiofalo *et al*., 2005) as regards fat and protein was similar to that of the Parma ham. The most represented fatty acids are: palmitic, stearic, oleic, and linoleic, similar to those observed in the Iberian ham, even though with some small differences (Cava *et al*. 2003) and in cured Parma ham (Bosi *et al*. 2000), Zanardi *et al*. 2000). In comparison with the Parma ham, the saturated class (SFA) content of Nero Siciliano ham was lower, similar levels were observed for the monounsaturated class (MUFA) and higher levels for the polyunsaturated class (PUFA), testifying the better nutritional characteristics of the Sicilian product. As a consequence, the unsaturated/saturated ratio was similar to that of Iberian ham and higher to that of Parma ham. The quality indices, Atherogenic and Thrombogenic, correlated with the cardiovascular diseases are lower in the ham of the black pig in respect to the white ones.

Perspectives and potentialities

The Nero Siciliano pig, above all on the Nebrodi, was the object of choral sympathy in the past: its meat and its derived products have always caused considerable, even titled, excellence, but its way of living, generally in an extensive condition, in complete freedom, has often provoked aversions to it, at times, even ferocious.

This happened when the production of the woods and underwoods, rather lacking, led the animal towards the search of food in the neighbouring inhabited centres, and, therefore to the damage of crops, and often even to the apprehension to man, with the result of a drastic vendetta of the same public Administrations that authorised the extermination, with great risks for this interesting TGA (Chiofalo, 2000 a; b).

The problem was presented, at the act of proposal for the forming of the natural Park of the Nebrodi, with the environmental component which saw with a certain preoccupation, and surely unwelcome, the presence of a wild pig in the territory of the Park. All this was in front of two potentialities of wealth: the environment with all its varied and unrepeatable beauty and the considerable reserve of animal biodiversity (Chiofalo, 1993): both also deserving great attention and especially in the interest of man. Therefore, there was the exigency to protect these two realities, a protection that could not be excluded from the evaluation of the existing ones.

This regarded, above all, the Nero Siciliano pig, with its interesting Nebrodi ecotype, known for its productive peculiarity as from very remote antiquity, for which it appeared necessary to pay attention to the exigencies of life, especially feeding, so as to prevent their excursions and damage, also aware of the fact that the wild system is not a biological equivalence as confirmed by the parameters of the metabolic-nutritional *status* examined by the researchers of the Zootechnical Section of the University of Messina (Chiofalo *et al*., 2001a; Liotta *et al*., 2001; Chiofalo *et al*., 2001b) which did not give complete tranquillity. These results would testify that the wild system is not in line with the platform of biological safety that only conditions of welfare can permit. As regards same it became opportune to sustain and to evaluate what is an existential characteristic of great significance for the Nero Siciliano pig, that is, its rusticity such as the attitude to live in quite difficult environmental conditions. Besides, it is a typical characteristic, as is known, of Mediterranean pigs, for example the Iberian pig, which would see them similar to the Napoletano-Casertana pig. The latter, accepted to be among the best in Italy (Marchi and Pucci, 1923) to point out the role that same would carry out in the field of Mediterranean pig populations. A pig, the Nero Siciliano, which has all the characteristics and the recognition as much genetic-constitutional as environmental, compared to the Iberian pig and, therefore, worthy of attention so as to safeguard and pilot in a more pertinent way the productive potentialities, involving in the protection of the animal, the maintenance of the genetic biodiversity, including that of the environment: see the Iberian pig as the pillar of an important ecosystem that finds sustenance in its own habitat, contributing to protect it, thanks to the success of its productions. In this regard, the anthropical presence is essential, as also Matassino (1996) underlines. Aromatic essences are seen in the natural flora of the Nebrodi (Liotta *et al.*, 2006b) and in certain Sicilian agricultural-sylvan-pastoral systems as well as in the Spanish “dehesa”, that contribute to render the meat of the pig esteemed and sought. In addition, the presence of the acorn and its importance in the feeding of the pig should be considered, as already mentioned, by Varrone in the “*de re rustica*”. The Iberian Jamon de bellota (acorn), especially the “Gran Riserva” Joselito production (the Ferrari of hams) very difficult to find outside Spain, produced with only black Iberian pigs (Uccella, 2000), can be considered an example. Besides, it is known that the Spanish have made a fortune with their Jamon, rich in oleic acid, and on the Iberian pig “Pata negra”, reared in freedom, so much so as to call it the Pata Negra, as Ernestina Ludeck, a key figure of the Pata Negra, mentions “aceite de oliva en cuatro patos”.

The recall to the acorn takes us to the woods of the Nebrodi mountains where, as has already been mentioned, numerous varieties of oaks with considerable productions of acorn, besides tubers, roots, mushrooms etc. are present available to the black pig. All this is connected to the meteorological trend of the seasons which, at times, puts the Nero Siciliano pig in great difficulty, with repercussions on the qualitative-quantitative productions, but also on its very existence.

Rearing outdore

As the value of this TGA is well-known also in its meaning of cultural patrimony and of the expression of life as well as of the territorial history as Matassino *et al.* (1993, 1996) and Matassino and Grasso (1996) have also pointed out many times, the TGA are the only ones to have the performances for a zootechnical role in determining difficult environments, with a programme financed by the European Community (Chiofalo, 2000c; 2001a; 2001b; Chiofalo *et al.*, 2002; 2004) it has been thought to overcome the climatic-seasonal-feeding uncertainty by making common feeds available (grains) and built shelters so that the animal could serve of them according to its necessity and autonomously, thus making up the conditions of the “*élevage en plein air*”: a premise for biological productions, thanks to the better life condition of the animals, and protecting at the same time the environment. This was done so as to analyse in a responding manner the potentialities of the animal in its natural environment, in line with a correct administration of the territory and, also, to guarantee the human consumer. In order to evaluate the responses, not only from a quantitative but also and, above all, from a qualitative point of view, we compared animals living in extensive conditions and those “*en plein air*” by determining the “*in vitam*” and “*post mortem*” performances. The observations (Liotta *et al.*, 2001) in a period that ranged from June to December had shown ADG (average daily gain) of 77 g for the animals in an extensive condition with maximum values in the month of October, whereas, for those “*en plein air*”, the ADG was, on average, 320 g with points that reached over 400 g.

In the animals of extensive conditions, the NEFAs (Chiofalo *et al.*, 2001a), which represent the parameter most sensitive to the energetic deficit, have shown significantly higher values during the most difficult period (Summer months) with ADG (Liotta *et al.*, 2001) at zero testifying the high energetic requirements, while the same values are low in the month of October when the living conditions of the animals improve.

In the months of July and August a considerable increase of glucose (Chiofalo *et al.*, 2001a) could be related to the situations of stress. This confirms that the extensive conditions are not reassuring as regards the conditions of the pig for the quality of productions with doubts also for human health.

For the pigs reared “*en plein air*”, besides the best ADG (Liotta *et al.*, 2001), the parameters of the energetic and protein metabolism, as well as the hepatic functionality (Chiofalo *et al.*, 2001a) and the quality indices (Atherogenic and Thrombogenic) (Chiofalo *et al.*, 2001b) are results of great interest.

Besides, the pigs kept in extensive conditions reached a weight of slaughter (70.88 Kg) at the age of 250 days, while those “*en plein air*” reached it (79.71 Kg) in 160 days, thanks, obviously, to the suitable rearing conditions (Liotta *et al.*, 2001). The yields at slaughter and the thickness of the dorsal lard were better in the plein air group than those in the extensive condition group, however, similar to those obtained for the different genetic types (Piccolo *et al.*, 1979; Campodoni *et al.*, 1999). Attention should be paid to the estimated percentage of lean cuts in the pigs in extensive conditions (Chiofalo *et al.*, 2004). The biometric data regarding the length of the half carcass and the thoracic depth for the animals “*en plein air*” are slightly higher (Liotta *et al.*, 2002). The reproductive performances are better as compared to those of the other autochthonous pigs of the Mediterranean area (Liotta *et al.,* 2006a) and in line with those observed by Matassino and Grasso (1996). As regards the chemical composition of the meat a significantly higher content of protein was observed in subjects of the plein air group (Zumbo *et al*., 2003)

As has already been mentioned, it has not been the passing from the extensive to the intensive conditions, but it has been to ensure the satisfaction of the basic exigencies of life, contemplating even on minimal conditions of feeding assistance and of shelters, respecting the usual environmental context (pasture and territory), trying to optimise the extensive-traditional system (Casabianca, 1996) with greater attention towards the animal: all this is done, as already mentioned, to sustain the rusticity of the animal in welfare conditions, as the fertility connected in a significant way to feeding (Chiofalo, 1981).

As regards the qualitative-organoleptic peculiarities of the products (meat-lard etc.) the most up-to-date and sophisticated methodologies of bio-chemical-physical evaluation, and the sensorial judgements performed with various panel tests (tests of tasting) carried out by different consumers, no differences were found between the two typologies of production.

The result has been that of paying more attention towards the Nero Siciliano pig in recent times, with considerable increases in the numerical consistence and the creation of a protection Consortium which has also been activated to obtain the official recognition of the quality of the meats and of the derived products, obtained from animals identified and reared in the open. As regards these animals, a Nebrodock type ham was produced, to be considered as a sort of local Pata Negra, and a typology of lard of great dietetic interest for its acidic composition, without speaking about other typical specialities *in itinere* .

Besides, as also Grasso *et al*. (1996) point out, it means paying adequate attention and looking towards the future, to the autochthonous genetic resources and to the characterisation of their productions with the implications on the development of the involved germ plasms, obviously, with all the possible cautions as regards the safeguard of the genetic variability (Gandini *et al*., 1996).

This has determined the boom of the black pig breeds, almost a recovery in comparison with the specialised breeds, noble and highly productive in the North, and it represents an opportunity to protect the genetic variability “in situ” considering that the black pig breeds are often found in natural agricultural-sylvan-zootechnical systems, whose best defence passes through the evaluation of the respective productions.

Moreover, the breeding “en plein air” would lead to the strengthening of the productivity also as an expression of the territory: the most important natural resource and the most interesting investment for the future.

Besides, the productivity of TGA in the internal areas that often goes towards delimitation, could assume a socio-economic significance of great importance also, as Christine de Sainte Marie *et al*. (1996) recall concerning pig breeding in Corsica, for the same presence of man. Therefore, the animal germ plasm is also an instrument of safeguard of the territory.

The productions of the Nero Siciliano pig could be identified as a typical niche product, with specificity and quality connected to the animal as well as the territory; a product marked, not by a repetitive static nature, but by the dynamic innovation leading to the pasture of an unpolluted environment which is renewed and to a genetic patrimony in a perennial future with the potentialities which are extended.

In reality, this means the protection of the genetic resource and the relative biodiversity, in that it is a historical cultural patrimony and a testimony of a past civilisation, for example, that of the farming civilisation**,** and looking at the autochthonous animal germ plasm as a formidable wealth of perspective.

The productions of the niche of these black pig populations with due measures of control in the theme of safety (traceability, recall) could be moved, as has already been said many times, in the optics of Mediterranean pig-breeding, not imitative or supplementary to the continental one, and neither in counter-opposition (Zucchi and Ferretti, 1999), but setting up, with a valid line of distribution, orientations of integrative and/or alternative production more to the measure of man (see the Mediterranean diet) with all the occurring guarantees (Chiofalo, 2000c).

For this type of pig-breeding, the Nero Siciliano pig has all the requisites to assume a role of protagonist and "L'élevage en plein air" experimented by us, could confirm that zootechny and environment constitute a binomial of extraordinary value, not in antagonism but in full synergy with the development and economy of the territory (Chiofalo, 1997), where the same conservation of the autochthonous animal germ plasm, beyond the cultural moment, will also become an important instrument for the administration of the territory.

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