Mangalitsa breed characterization for *in vitro* germoplasm preservation program

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Abstract
The aim of this research was to characterize Red Mangalitsa breed population from Romania in order to include it in germoplasm preservation program. For this purpose we have chosen genetic characterization by individual fingerprinting using 14 microsatellite markers and also biochemical characterization of carcass quality by fatty acid level determination. Microsatellite markers helped us to characterize the population and give scientific base for management practices. The fatty acid determination has indicated a higher level of polyunsaturated fatty acids, especially linoleic acid and a higher P/S (polyunsaturated fatty acids / saturated fatty acids) ratio. These results confirm that the analyzed population has Red Mangalitsa characteristics and could be use to long germoplasm preservation.

Keywords: characterization, fatty acid, Mangalitsa, microsatellite markers

1. Introduction

Preservation of the genetic variation is considered to be a very important tool to avoid irrecoverable loss of rare breeds or genes, to re-establish a breed, or to support breeding in small populations [1]. In order to the criteria provided by Ruane and Sonino in 2006 [2] for choosing a specific breed for a preservation program, in Hungary Mangalitsa breed had beneficed a large commercial project that increased the number of sows and the amount of Mangalitsa products too [3]. Beside Hungary and Romania there are mentionable populations of Mangalitsa in Austria, Germany and Switzerland and also in the area of the formed Yugoslavia [4].

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For genetic distance characterization of animal breed, Food and Agriculture Organization (FAO) and International Society of Animal Genetics (ISAG) recommend to use microsatellite markers [5]. The genetic relationships among the indigenous Hungarian Mangalitsa swine breeds have been studied by ten microsatellite markers in order to characterize the population [6]. On the other study, the genetic distance between Mangalitsa and Duroc breeds is more than that between Iberian and Duroc [7].
Because Mangalitsa is included into the fat type breeds, it is important to know the fatty acids composition and the cholesterol content of lard and intra muscular fat for biochemical quality characterization [8]. The fat of Mangalitsa contains 12-16% less saturated fatty acids and 8-10% more unsaturated fatty acids than modern pig breeds [9].
Old knowledge of Mangalitsa breeding has to be upgraded, new physiological data had to be

collected and new technologies for feeding and housing had to be established with special regard to old values of this pig breed and to increase the profit rate of breeders [3].

2. Materials and methods

For genetic characterization a total number of 120 individual blood samples from Red Mangalita were collected from 110 females and 10 males from the Roman and Turda populations (S.C. Suinprod S.A. Roman and Agricultural Research and Development Station Turda).
The selection of microsatellite markers for DNA fingerprinting was performed at ISAG-FAO recommendation, 14 microsatellites being analyzed in the present study: SO005, SO090, SO101, SO155, SO227, SO228, SO386, SW24, SW72, SW240, SW857, SW911, SW936 and SW951. Porcine genomic DNA was extracted from blood, while PCR conditions included multiplexes and annealing condition can be found at http://www.toulouse.inra.fr/lgc/pig/panel.html. Genotyping was done with ABI Genetic Analyzer by fluorescent fragment analysis.
Results were analyzed by Genepop 1.2 software [10], in order to determine the number of alleles per locus, genotype distribution (expected and observed number of heterozygote using Levene`s correction), allele frequencies and inbreeding coefficients (according to Weir and Cockerham, 1984 and also Robertson and Hill, 1984).
For biochemical characterization there were slaughtered 20 castrated male pigs averaging 96 kg. The samples of ham and back fat were analyzed by gas chromatography to determine fatty acids level. Peak areas of identified fatty acids were used to calculate the relative percentage fatty acid composition of the total fatty acids.
The percentage of saturated fatty acids (SFA) was calculated by adding C12:0, C14:0, C18:0 and C20:0. Unsaturated fatty acids (UFA) were represented by monounsaturated fatty acids (MUFA), consisted of C16:1 and C18:1 and polyunsaturated fatty acids (PUFA) calculated by summing the remaining fatty acids (C18:2, C18:3, C20:4, C20:5 and C20:6). To assess the nutritional properties of Mangalitsa meat the PUFA/SFA ratio (P/S) and n-6/n-3 PUFA ratio were determinate.

3. Results and discussion

A total of 66 alleles were detected across the 14 analyzed loci. All loci were polymorphic and the number of alleles have varied between 3 (SO227 and SW951) and 8 (SO005) with a mean value and their standard error (SE) of 4.714 ± 0.339. A relatively low number of alleles (3.3) was obtained although by Garcia et al. in 2006 on Mangalitsa used 27 microsatellite markers, while the number of alleles of Duroc breed was 6.6 and to Iberian breed 8.4 [7]. The average number of effective alleles was 2.849 ± 0.286 in comparison with 1.8 obtained by Garcia et al. in their research [7].
The observed hetetozygosity (Ho) varied between 0.092 (SO227) and 0.882 (SW857), with a mean and SE of 0.626 ± 0.059. The expected heterozygosity (He) value was 0.589 ± 0.050 while the unbiased expected heterozygosity (UHe) presented a mean value of 0.592 ± 0.051.
Principal Coordinates Analysis (PCA) shows a homogenate population (figure 1). Although, the excess of observed heterozygosity based on calculated fixation index (F_{IS}) is characteristic to gene immigration in the analyzed population (-0.053 ± 0.017). In this case, the incoming of individuals from foreign population could be explained by the import of animals from Hungary and Austria realized in the last years to keep out the genetic drift.

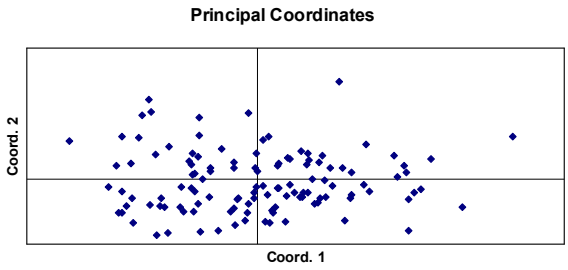

Fig. 1. Principal Coordinates Analysis of Mangalitsa

The results of fatty acids profiles of intramuscular and back fat lipids are shown in figure 2. In relation with the individual fatty acids, Mangalitsa shows a higher level of palmitic and stearic (SFA), oleic (MUFA) and linoleic (PUFA). The value of linoleic acid was double than that found by the other researchers in the same breed [8].
The intramuscular and back fat lipids have a higher percent of UFA (64.41). Our results indicated similar distribution with that reported by

Szabo and Farkas (2005) at back fat of Red Mangalita (UFA: 61.33% vs. 63.01%). In an other study, Holló et al. (2003) showed that in Blond Mangalita, meat (*musculus longissimus dorsi*) has an UFA percent of 68.97 and 67.58 in *semimembranosus* muscle, while we was finding a similar level of UFA in ham (67.54%). Our results are better even in comparison to the data of UFA in ham than that found by Fernandez et al. (2007) in five varieties of Spanish ham (57.41 - 59.06% in Serrano and Teruel hams and 64.60 - 65.01% in Iberian hams).

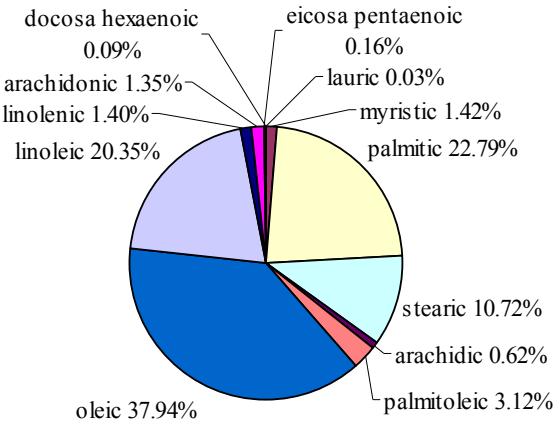

Fig. 2. Fatty acids level in ham and back fat

In relation with P/S, a value above 0.4 is recommended for healthy foods and diets, but just a high proportion on PUFA is not necessarily healthy if it is not balanced in relation with the n-6/n-3 ratio [11]. From this point of view, the Mangalitsa has a P/S ratio of 0.66 (with a better value of intramuscular fat than back fat) higher than that found by Fernandez M. et al. (2007) at "Jamón Serrano" (0.3). Otherwise, the n-6/n-3 ratio was 13.12, more balanced than that determinated by Szabo P. at Blonde Mangalitsa.

4. Conclusions

In the present study Red Mangalitsa breed was characterized by molecular and biochemical instruments. Using 14 microsatellite markers it was possible to view a homogenate population, with a higher level of observed hetetozygosity than the expected one, as a result of some gene immigration (Mangalitsa import from other population). The fatty acid level on intramuscular and back fat lipid had high content of PUFA and the very good P/S and n-6/n-3 ratios benefic from human nutrition point of view. The results of Red Mangalitsa characterization serve as a basis for future applications of *in vitro* germoplasm preservation.

Acknowledgements

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