Detection of ryanodine receptor mutation in Mangalitsa crossbred pigs bred in Transylvania

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Abstract. The diversity of ryanodine receptor (Ryr-1) gene in Transylvanian Mangalitsa pure and crossbred swine was evaluated through real time polymerase chain reaction (RT-PCR). A total of 167 animals, aged between 5-12 months were tested and 4 animals expressed the pale soft exudative (PSE) phenotype. Genotyping also revealed that the proportion of heterozygotes (C/T) varies substantially ($P< 0.05$) among the purebred Mangalitsa and the crossbred ones. Compared to crossbred Mangalitsa, the purebreds revealed the highest percentage (73.5%) of homozygous individuals for the normal C/C allele at Ryr-1 locus. The results obtained prove the fact that expression of PSE meat in Mangalitsa purebred is unlikely to occur, but when crossbred with other breeds such as Duroc and Landrace the genetic testing is imperious.
Key Words: homozygotes, diversity, genotype, meat, RT-PCR.

Introduction. Breeding of domesticated animals for food production historically was directed to increase productive performances (Janovic et al 2005) becoming important to highly select individuals. Focusing selection strictly on one area of performance, such as meat quality, while disregarding potential negative effects on growth or carcass quality could actually create a negative trend for the overall economic performance of the animal (Barbut et al 2008). Genotype by environment interactions are particularly relevant to swine improvement programs, including genetics of meat quality, as the progeny of individuals raised, tested and slaughtered under intensive raising conditions have to adjust to varying commercial production and plant environments (Bijma & Van Arendonk 1998; Brandt & Taubert 1998; Lutaaya et al 2002; Van der Werf et al 1994). Due to the fact that in Romania the pig breeding has suffered greatly from the manifestation of pale, soft, exudative (PSE) meat on the slaughter line, it is important that a selection program should be implemented against this manifestation in these production units. Researches concerning the factors which contribute to PSE meat date back many years and are still being performed underlining a persistency of the problem. A major factor that can determine the development of PSE cases in pork is characterized as "Porcine Stress Syndrome" (PSS). This syndrome has been associated with a recessive mutation in the gene coding for porcine calcium release channel, also called the ryanodine receptor gene (Ryr-1 locus) or halothane gene (Hal) (Fujii et al 1991) which is located on chromosome 6 (Harbitz et al 1990). This single nucleotide substitution (T/C) in the gene encoding the skeletal ryanodine receptor 1 (Ryr1) allowed the accurate diagnosis on the basis of three types: normal, heterozygote and homozygote (Fujii et al 1991). Based on this fact, the halothane gene is the most studied major gene affecting meat quality, and it is the first practical manipulation of a major gene in pig breeding using molecular biology tools (Fujii et al 1991; Lister 1987; MacLennan & Philips 1992; Otsu et al 1992). Mangalitsa pigs were brought to Romania from Serbia in the 19th century (Transylvania - 1833; Oltenia, 1860) (Gligor et al 1969). This breed is appreciated for high quality of meat, used for preparing special local products (Ciobanu et al 2001). An increasing number of units have

started to use Mangalitsa schemes involving Duroc and Landrace in preparing a traditional old type of bacon, very appreciated by the Eastern European market. Because no genetic testing is used when breeding these swine in our national system, our study focused on analyzing the genetic background for ryanodine mutation occurrence in the population of Mangalitsa in Transylvania region by using Real-time PCR technique.

Material and Method. A total of 53 purebred Mangalitsa and 114 Mangalitsa crossbred pigs (Duroc n = 54, Landrace n = 60), aged between 5 -12 months, were tested for ryanodine mutation following the harvesting of blood samples from auricular veins. The blood was collected into sterile tubes containing K3EDTA as anticoagulant and stored at 4°C until further analysis. All the samples were collected from the north-west region of Transylvania.

Genomic DNA extraction. The DNA extraction, followed the steps previously reported by Balteanu et al (2010), i.e. two hundred microliters of blood were washed three times with phosphate buffered 4 saline (0.5 M). After the final wash the cell pellet was incubated 15 min at 95°C in 50 µL of 200 mM NaOH solution and then neutralized with 50 µL of 200 mM HCl + 100 mM Tris HCl (pH = 8.5) solution. The DNA quantity and purity of each sample were assessed on a Nanodrop ND-1000 spectrophotometer analyser (NanoDrop Technologies, Inc., 70 Wilmington, DE, USA).

Real Time PCR genotyping. All the samples tested were screened for ryanodine receptor mutation using a set of primers previously described by Burgos et al (2005) and (Burgos et al 2005), i.e. PIGRYR1F (5'- CCCTGTGTGTGTGCAATGG-3') and PIGRYR1R (5'-GTTTGTCTGCAGCAGAAGCT 3') that amplified a 95 bp fragment of the pig RYR1 gene. The probes used were PIGRYR1V2 (labeled with VIC dye) and PIGRYR1F2 (labeled with FAM). Each PCR reaction mix (25 µL) comprised: 1X PCR green Buffer, 2.5 mM MgCl2, 5 pmol of each primer, dNTPs each at 200 µM, 2.5 U of Taq DNA Polymerase (Promega, Madison, WI, USA) and 100 ng of genomic DNA. PCR was performed on a CFX Connect thermal cycler (BioRad, USA), under the following conditions: 95°C for 10 min followed by 40 and 1 min at 62°C. The data collected and the melting curve analysis was carried out using the CFX ManagerTM software.

Statistical interpretation. The allelic frequencies were calculated according to a previously reported method (Falconer 1982). The OriginPro (Software version 8.5, Origin Lab Institute, USA) was used for the ANOVA one-way and the least significant difference test when comparing the genotype frequencies.

Results and Discussion. The allelic distribution of some of the samples tested is shown in Figure 1.

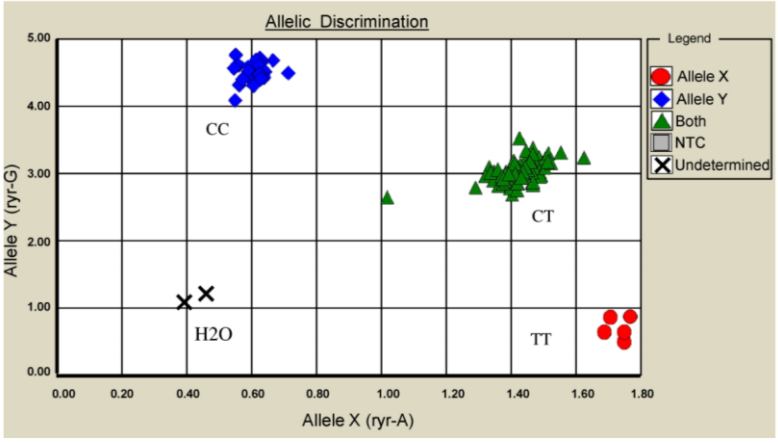

Figure 1. Ryr-1 genotypes' identification through RT-PCR. Diamonds - CC pigs; Triangles - CT individuals; circles - TT individuals.

The frequency of the desired C allele was similar among purebred Mangalitsa and 95 crossbred Mangalitsa while the proportion of heterozygotes (C/T) varied substantially (P<96 0.05). Purebred Mangalitsa samples showed fewer heterozygotes (26.41%) while the crossbreds showed higher percentages (52.63%). The highest frequency of T allele was noticed in Landrace – Mangalitsa crossbred samples, where 6% were found to be C/C genotype. Although descended from wild boar and considered a primitive breed with much less genetic mutations, some of the Mangalitsa pigs purebred swine tested revealed to carry C allele in heterozygous state. The majority of the samples (n = 47) (88.67%) revealed the C/C genotype (Table 1).

Table 1

Distribution of ryr-1 genotype and the allele frequencies in the Mangalitsa swine population studied

Breed	*N*	*Genotype*[a]			*Allele frequencies*	
		C/C	*C/T*	*T/T*	*C*	*T*
Mangalitsa	53	39 (73.58)[b]	14 (26.41)[b]	0	0.847	0.153
Duroc x Mangaitsa crossbred	54	18 (33.33)[b]	36 (66.66)[b]	0	0.849	0.151
Landrace x Mangalitsa crossbred	60	32 (53.33)[b]	24 (40)[b]	4 (6.66)[b]	0.814	0.189
Total	167	89 (53.29)[b]	74 (44.31)	4 (2.39)	0.843	0.157

[a]Genotype determined by RT-PCR assay for ryr-1 locus; C/C = normal pig; C/T = 223 heterozygote; T/T = homozygote mutant.
[b]Percentage of pigs with breed is given in parentheses.

Although several reports demonstrated the absence of the stress gene in the local pig population from Central and Eastern Europe (Sarac et al 1998). Ciobanu et al (2001) reported the absence of the T allele in Mangalitsa breeds in Transylvania in 2001; therefore it seems that T allele has been introgressed by crossbreeding of Managalitsa breed with other breeds. This fact is 6 probably due to the limited use of artificial insemination in small-scale Mangalitsa production farms and therefore the certified material is not widely used. Other previous studies related to Ryr-1 gene mutation and meat quality have been conducted (Obi et al 2010) revealing that also the heterozygote genotype is likely to develop PSE meat. It was that CT genotype more frequently produces PSE meat than pigs with normal genotype (Pommier & Houde 1993; Cheah et al 1995; Horiuchi et al 1996). Other studies (Houde et al 1993) showed that the proportion of heterozygotes varies substantially among breeds raised in Canada, the Duroc breed containing the fewest heterozygotes and the Landrace the most. Researches on Duroc, Large White and Landrace breeds raised in China (Ruan et al 2013) revealed also a number of heterozygotes and several undesirable TT homozygotes. However, the Ryr-1 allelic frequencies in our study showed that Landrace x Mangalitsa and Duroc x Mangalitsa crossbreds are carriers in a high percentage, while Mangalitsa purebreds have the lowest carrier frequency.

Conclusions. Having in mind that the ryanodine receptor gene is considered for PSE expression in meat, the results obtained show that there is a low probability of occurrence in primitive breeds such as Mangalitsa. However, frequent crossbreeding of Mangalitsa with Duroc and Landrace swine for improving the meat quantity and lowering the fat content can lead to the spread of mutant gene carriers. Our observations indicate that a continuous selection in Mangalitsa crossbreds is required to fix the favorable alleles and decrease the T/T genotype frequency. We further suggest that a continuous selection of the Ryr-1 locus be made by the Transylvanian Mangalitsa swine breeders to establish the PSE-free lines populations.

References

Balteanu V. A., Pop F. D., Vlaic A., Carsai T. C., Creanga S., Rusu A. R., 2010 Characterization of the αS1-casein I allele provides evidence for phylogeny of the ancient Romanian Grey Steppe cattle Moldavian strain. Scientific Papers, Animal Husbandry, USAMV Iasi 53:315-320.

Barbut S., Sosnicki A. A., Lonergan S. M., Knapp T., Ciobanu D. C., Gatcliffe L. J., Huff Lonergan E., Wilson E. W., 2008 Progress in reducing the pale, soft and exudative (PSE) problem in pork and poultry meat. Meat Sci 79:46-63.

Bijma P., Van Arendonk J. A. M., 1998 Maximizing genetic gain for the sire line of a crossbreeding scheme utilizing both purebred and crossbred information. Animal Science 66:529-542.

Brandt H., Täubert H., 1998 Parameter estimates for purebred and crossbred performances in pigs. Journal of Animal Breeding and Genetics 115:97–104.

Burgos C., Carrodeguas J. A., Moreno C., Sanchez A. C., Tarrafeta L., Barcelona J. A., Buesa P. L., 2005 A real time PCR (RT-PCR) alternative assay to detect the T/C mutation in position 1843 of the ryanodine receptor gene. Meat Sci 70:395–398.

Cheah K. S., Cheah A. M., Krausgrill D. I., 1995 Variations in meat quality in live halothane heterozygotes identified by biopsy samples of *M. longissimus dorsi*. Meat Sci 39:293-300.

Ciobanu D. C., Day A. E., Nagy A., Wales R., Rothschild M. F., Plastow G. S., 2001 Genetic variation in two conserved local Romanian pig breeds using type 1 DNA markers. Genet Sel Evol 33:417-432.

Falconer D. S., 1982 Introduction to quantitative genetics. 2nd edition, Longeman, New York.

Fujii J., Otsu K., Zorzato F., De Leon S., Khanna V. K., Weiler J. E., 1991 Identification of mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science 253:448–451.

Gligor V., Radu A., Stanciulescu M., 1969 Zootehnia Romaniei - Porcine. Editura Academiei, Bucuresti, pp. 45-52.

Harbitz I., Chowdhary B., Thomsen P. D., Davies W., Kaufmann U., Gustavsson I., Christensen K., Hauge J. G., 1990 Assignment of the porcine calcium release channel gene, a candidate for the malignant hyperthermia locus, to the 6p11-q21 segment of chromosome 6. Genomics 8:243-248.

Horiuchi A., Kawarasaki T., Chikyu M., Sone M., Noguchi H., 1996 Relationship between skeletal muscle ryanodine receptor (RyR1) genotypes and meat quality of commercial pork loins. Anim Sci Technol 67:387-392.

Houde A., Pommier S. A, Roy R., 1993 Detection of the ryanodine receptor mutation associated with malignant hyperthermia in purebred swine populations. J Anim Sci 71:1414-1418.

Jovanović S., Trailović R., Savić M., Sarač M., 2005 Porcine stress syndrome (PSS) and ryanodine receptor 1 (Ryr1) gene mutation in European wild pig (*Sus scrofa ferus*). Acta Veterinaria Beograd 55:251-255.

Lister D., 1987 The physiology and biochemistry of the porcine stress syndrome. Martinus Nijhoff Publishers, Boston, USA.

Lutaaya E., Misztal I., Mabry J. W., Short T., Timm H. H., Holzbauer R., 2002 Joint evaluation of purebreds and crossbreds in swine. J Anim Sci 80:2263–2266.

Maclennan D. H., Philips M. S., 1992 Malignant hyperthermia. Science 256:789–794.

Obi T., Matsumoto M., Miyazaki K., Kitsutaka K., Tamaki M., Takase K., Miyamoto A., Oka T., Kawamoto Y., Nakada T., 2010 Skeletal ryanodine receptor 1-heterozygous PSE (pale, soft and exudative) meat contains a higher concentration of myoglobin than genetically normal PSE meat in pigs. Asian-Aust J Anim Sci 23:1244–1249.

Otsu K., Phillips M. S., Khanna V. K., De Leon S., Maclennan D. H., 1992 Refinement of diagnostic assays for a probable causal mutation for porcine and human malignant 162 hyperthermia. Genomics 13:201–214.

Pommier S. A., Houde A., 1993 Effect of the genotype for malignant hyperthermia as determined by a restriction endonuclease assay on the quality characteristics of commercial pork loins. J Anim Sci 71:420-425.
Ruan G. R., Xing Y. Y., Fan Y., Qiao R. M., He X. F., Yang B., Ding N. S., Ren J., Huang L. S., Xiao S. J., 2013 Genetic variation at RYR1, IGF2, FUT1, MUC13, and KPL2 mutations affecting production traits in Chinese commercial pig breeds. Czech J Anim Sci 58:65–70.
Sarac M., Jovanovic S., Gagrcin D., 1998 Distribution of the allele frequencies for some polymorphic enzyme and protein systems in two Yugoslav autochtonal pig breeds: Moravka and Mangulica. In: Proceedings in DAGENE Conference, Budapest, Hungary, pp. 112-115.
Van Der Werf J. H. J., Van Der Wei M., Brascamp E. W., 1994 Combined crossbred and purebred selection to maximize genetic response in crossbreds. In Proceedings of the 5th World Congress on Genetics Applied to Livestock Production, Ontario, Canada, 7-12 August, pp. 234-321.

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