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Genetic variability in myostatin (MSTN) and ryanodine (RYR1) receptor genes in crossbred pigs Youna x Pietrain

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Abstract

Stoykova-Grigorova, R., Stefanova, K. & Bozhilova-Sakova, M. (2023). Genetic variability in myostatin (MSTN) and ryanodine (RYR1) receptor genes in crossbred pigs Youna x Pietrain *Bulg. J. Agric. Sci., 29 (2)*, 378–383

The aim of present study was investigation of genetic variability in myostatin and ryanodine receptor genes in pigs. 26 male and female crossbred pigs Youna x Pietrain were studied, they were part of the Institute of animal science – Kostinbrod, Bulgaria. Genomic DNA was extracted from hair by manual purification kit. PCR amplification and RFLP analysis were carried out. Specific restriction enzymes were used as fallowed: *TaqI* for MSTN/exon3, *DraI* for MSTN/promoter and *HhaI* for RYR1 locus. The fragments were separated by agarose electrophoresis and the obtained results were visualized under UV light. Based on results, promoter of MSTN and RYR1 genes were found to be polymorphic while the exon 3 of MSTN gene was monomorphic. The allelic frequencies in MSTN/promotor region were 0.96 for allele *T* and 0.04 for *A* and the genotype frequencies were 0.92 and 0.08 for *TT* and *TA*, respectively. Homozygous genotype *AA* was not found in this herd. In MSTN/exon3 region only allele C and genotype CC were observed. In this population RYR1 locus showed low level of polymorphism. The allelic frequencies were 0.98 for allele N and 0.02 for allele n. Genotype frequencies were 0.96 for homozygous dominant genotype NN and 0.04 for heterozygous genotype Nn. Homozygous recessive genotype nn was not found. Observed (Ho) and expected (He) heterozygosity were 0.076 and 0.077 for MSTN/DraI and 0.038 and 0.039 for RYR1, respectively.

Keywords: PCR-RFLP; myostatin gene; ryanodine receptor gene; crossbred pigs Youna x Pietrain

Introduction

Large number of genes controls development of muscles and meat traits. One of the most important regulators of growth is myostatin (MSTN) (Sonstegard et al., 1998).

Myostatin or growth differentiation factor-8 (GDF-8) is a member of the mammalian growth transforming family (TGF-beta superfamily), which plays a role in the regulation. Its presence was found in skeletal muscles in pigs and cattle. (McPherron et al., 1997; Ji et al., 1998). In pigs the MSTN gene is located on chromosome 15 and it consists of two introns and three exons and it encodes the synthesis of glicoproprotein which is expressed in skeletal muscles (Bellinge et al., 2005). Changes in structural level of MSTN could influent on the development and meat quality (Cieslak et al., 2003). According to some authors (McPherron et al. 1997; Stratil and Kopečny, 1999; Cieslak et al., 2003) during the muscle growth MSTN inhibits the protein synthesis. Mutations within myostatin gene led to muscular hypertrophy and double muscling (Hanset, 1991; Williams, 2008). Such a major effect of a single gene defines it as candidate gene for improving of meat quality and quantity in farm animals (Rybarczyk et al. 2010).

Selection of pigs was directed to increase the percentage of lean meat but this action led to misbalance between quality and quantity of meat in Pietrain and Landrace. Thos phenomenon is known as porcine stress syndrome (PSS) or malignant hyperthermia (MH). Great number of authors

announced that pigs with PSS have high level of muscle tissue which leads to different changes in meat traits (Angelov, 1995; Monin, 1994; Marinova et al, 1995; Lee et al., 1999, Fisher et al., 2000). Halothane sensitivity in pigs is highly associated with some genetic markers in pigs. It was defined that halothane induced malignant hyperthermia (MH) is controlled by recessive alleles on RYR1 locus. PSS is genetically-depended on mutation in this gene which is used as candidate gene for high muscling in pigs (De Vries et al., 2000; Hamilton et al., 2000; Pedersen et al., 2001; Jovanovij et al., 2005; Davalos-Aranda et al., 2010).

RYR1 is part of a linkage group located on chromosome six of pig genome (Didion et al., 2000; Hebinck et al., 2000; Kim et al., 2001; Kuiper et al., 2001; Davoli et al., 2002). It produces two alleles – dominant allele *N* and recessive allele *n*. Stress sensitive animals were carrying the recessive genotype *nn* (Tăbăran et al., 2000).

The hybrid pigs Youna are desirable for the meat-producing sector, as they are characterized by lean meat. Pietrain breed is highly productive and the carcass is demonstrated lean meat, and high percentage of mutant allele in RYR1 gene and porcine stress syndrome (PSS) as well (De Vries et al., 2000). Number of authors reports studies on the influence of the paternal Pietren breed on the composition and quality of meat in hybrid pigs (Čechova et al., 2008; Czyzak-Runowska et al., 2015; Fisher et al., 2000; Škrlep et al., 2010). According to the French Pig Breeding Agency (2009), crossbreeds of Youna x Pietrain have PSE meat, which has a lower intramuscular fat content and a higher percentage of exudate loss in *m. Longissimus dorsi*.

The aim of the present study was to identify allele diversity of myostatin and ryanodine receptor genes in order to determine effective alleles influencing meat quantity and quality traits in pigs.

Materials and Methods

This study included 26 crossbred pigs Youna x Pietrain (7 males and 19 females), raised in Institute of Animal Science – Kostinbrtod, Bulgaria.

The samples were collected from the back of the animals (50 – 60 hairs of each animal) and stored at +4°C in sterile container.

The genetic diversity of myostatine (MSTN) and ryanodine receptor (RYR1**)** genes was estimated by PCR-RFLP method.

The analysis was performed in laboratory part of the AgroBioInstitute in Sofia, Bulgaria. It consisted the following steps:

DNA extraction

The genomic DNA was extracted from hair bulb by commercial kit Blood-Animal-Plant DNA Preparation Kit (Jena Bioscience) according to the provided instructions. The quality of the extracted DNA was determined by UV-spectrophotometer.

Two regions of myostatin gene were investigated: first one included the promotor region and the second one included the exon 3.

For this study, it was used specific sets of primers (Table 1) and 3 specific endonucleases *TaqI* for exon 3 and *DraI* for promotor region of MSTN gene and *HhaI* for RYR1 gene (Table 1).

Polymerase chain reaction (PCR)

The reaction mix was prepared by "ready to use" kit with final volume 15 µl (MyTaq HS Mix – Bioline), it contained all components needed: Taq polymerase, buffer, dNTPs, MgCl2, ddH2O. PCR amplification was carried out in Mastercycler-Eppendorf at specific conditions shown in Table 2. Identification of amplified fragments of MSTN and RYR1 genes was performed by agarose electrophoresis on 1.8% and 2% agarose gels stained by RedGel.

Restriction fragment length polymorphism (RFLP analysis)

The RFLP analysis was carried out with three different specific restriction enzymes described in Table 3.

The fragments length after RFLP analysis were identified using agarose electrophoresis on 3 and 3.5% agarose gel for exon 3 and promotor region of MSTN, respectively and on 2.5% agarose gel for RYR1 gene. The results were

Table 1. Locus, region, primers and length of PCR fragment of the two genes

Locus	Region	Primers	PCR product	References
MSTN	Exon 3	F: 5'-CTG CCT CTC TCT CTC TTC TCT GTC CTC-3 R: 5'-CTT TTT ATT GTA TGA TTT GTT TTG ATG-3'	899 bp	Cieslak et al. (2003)
	Promotor region	F: 5 ,-TTT TTG AGG AAA AAG ACA TTT CAA-3' R: 5'-ACA ACT TGC CAC ACC AGT GA-3'	397 bp	
RYR1		F: 5'-TCC AGT TTG CCA CAG GTC CAT ACC A-3' R: 5'-ATT CAC CGG AGT GGA GTC TCT GAG-3'	659 bp	Bašić et al. (1997)

Table 2. Condition of PCR amplification of the investigated genes

Locus	Primary denaturation	Cycles	Denaturation	Annealing	Elongation	Final elongation	Store
MSTN promotor region	95ºC/2 min	35	94ºC/45 s	57ºC/45 s	72ºC/60 s	72ºC/10 min	4ºC
MSTN (exon 3)	95ºC/3 min	35	95ºC/60 s	55ºC/60 s	72ºC/45 s	72ºC/3 min	4ºC
RYR1	94ºC/3 min	34	94ºC/60 s	57ºC/40 s	72ºC/10 s	72ºC/3 min	4ºC

Table 3. Conditions for RFLP analysis for MSTN and RYR1 genes

Locus	Enzyme	Time	Temperatufre	Place
MSTN-exon 3	TaqI	2 h	60°C	Thermocycler
MSTN-promotor	DraI	4 h	37°C	Thermal-block
RYR1	HhaI	4 h	37°C	Thermal-block

visualized under UV light after staining with fluorescence stain GelRed. The genotypes were determined according to the number and length of the restriction fragments. For work that is more accurate it was used DNA control – DNA Ladder, 100 bp (Jena Bioscience).

Statistical analysis

The statistical analysis of results was performed using soft wear GENAlex.

Results and Discussion

After PCR amplification in all 26 animals the PCR products were with expected length of 899 bp and 397 bp for MSTN/exon 3 and MSTN/promotor regions, respectively (Figures 1a and 1b).

After RFLP analysis in the two investigated regions of MSTN gene, the promoter region was found to be polymorphic, while the exon 3 of MSTN gene was monomorphic (Figures 2 and 3). The allelic frequencies in MSTN/promotor region were 0.96 for allele T and 0.04 for A. Genotype frequencies were 0.92 and 0.08 for *TT* and *TA*, respectively. Homozygous genotype *AA* was not found in this herd (Figure 2). MSTN/exon3 region was monomorphic, only allele C and genotype *CC* were observed. Observed (Ho) and expected (He) heterozygosity for MSTN/DraI were 0.076 and 0.077, respectively (Figure 3).

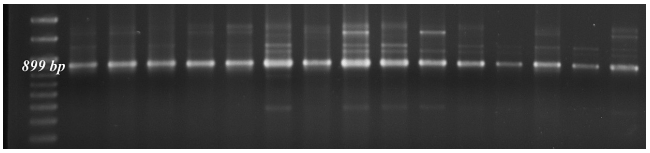

Fig. 1a. PCR products of MSTN/exon3 gene, after amplification, visualized in 1.8% agarose gel

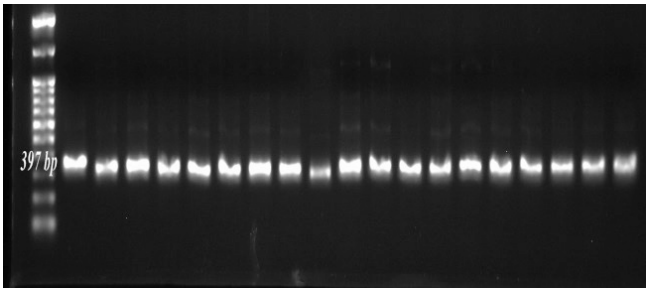

Fig. 1b. PCR products of MSTN/promoter gene, after amplification, visualized in 1.8% agarose gel

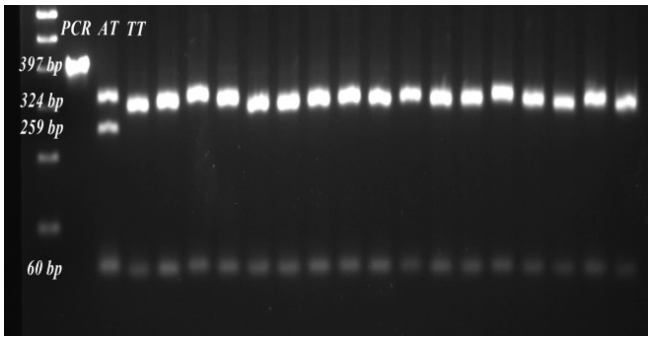

Fig. 2. Results of PCR-RFLP analysis of MSTN/promoter gene, segment digested with the restriction endonuclease DraI, visualized in 3% agarose gel

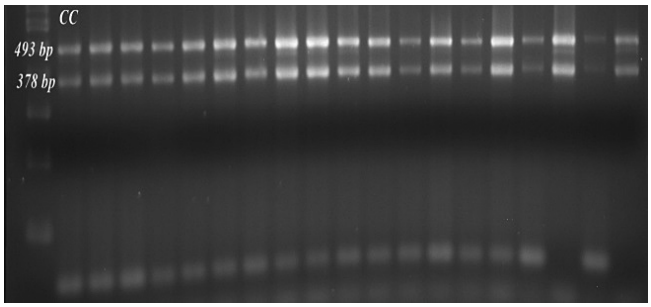

Fig. 3. Results of PCR-RFLP analysis of MSTN/exon3 gene segment digested with the restriction endonuclease TaqI, visualized in 3.5% agarose gel

For the first time polymorphism in MSTN gene promoter region was detected and described by Stratil & Kopečny (1999). The authors determined that all tested animals from breeds Pietrenne, Duroc and Hampshire were homozygous for allele *T*. In other three breeds Big White, Landrace and Meishan the frequency of allele *A* was 0.05-0.21. Results of the present study are in concordance with the above-mentioned paper.

Cieślak et al. (2003) reported similar results. Totally 294 randomly selected animals of eight breeds or crosses were analysed for DraI polymorphism in the myostatin promoter region. Except for one heterozygous individual of Large White x Pietrain and two of Torhybi line all remaining animals were TT homozygotes.

Other authors (Guimaraes et al., 2007) reported some polymorphisms in intron regions of MSTN gene in different pig breeds.

Porcine myostatin is an object of growing interest. Some mutations in porcine GDF8 gene were found, however their occurrence was very low (Stratil and Kopečny, 1999; Cieślak et al. 2003 and present study) indicating that myostatin protein is more stable in this species than in cattle. The presented results confirmed previous observations that porcine GDF8 is much more conserved and stable than bovine. According to Cieślak et al. (2003) the genotype frequency in GDF8 exon3 determined by TaqI restriction enzyme can be confusing. The authors observe the highest frequency of *TT* homozygotes only of synthetic line pigs, where the muscle fibre number per unit area was 29% higher if compared with Large White × Polish Landrace crosses due to muscle hyperplasia (Klosowska et al., 1998). However, the statistical analysis did not reveal any differences in phenotypic traits between the pigs of three genotypes. Although the C→T mutation does not change a type of encoded amino acid (Stratil & Kopečny, 1999), it can influence mRNA stability. Jiang & Gibson (1999) suggested a similar effect of silent mutation in leptin gene.

After PCR amplification of RYR1 gene in all 26 tested animals a fragment of 660 bp was amplified (Figure 4).

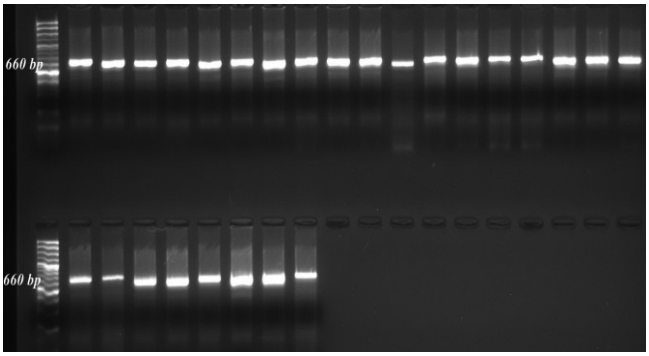

Fig. 4. PCR products of RYR 1 gene, after amplification, visualized in 2 % agarose gel

After RFLP analysis two genotypes were determined – homozygous genotype *NN* with two fragments – 494 bp and 166 bp and heterozygous genotype *Nn* with three fragments – 660 bp, 494 bp and 166 bp (Figure 5). In this population RYR1 locus showed low level of polymorphism. The allelic frequencies were 0.98 for allele *N* and 0.02 for allele n. Genotype frequencies were 0.96 for homozygous dominant genotype *NN* and 0.04 for heterozygous genotype *Nn*. Homozygous recessive genotype *nn* was not found. Observed (*Ho*) and expected (*He*) heterozygosity were 0.038 and 0.039, respectively. This results, may be explained by conservation and breeding strategies, which have been carried out.

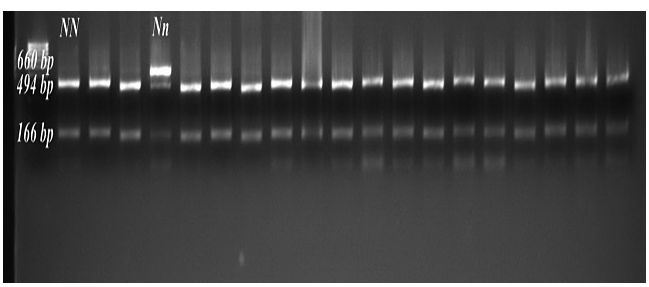

Fig. 5. Results of PCR-RFLP analysis of RYR 1 gene segment digested with the restriction endonuclease *HhaI,* visualized in 2.5% agarose gel

Table 4. Allele frequencies in MSTN/GDF8 promoter, MSTN/GDF8-exon3 and RYR 1 polymorphic sites in the studies pigs (n = 26)

Locus	Allele frequencies		Genotype frequencies			Heterozygosity	
MSTN/*DraI*	*A*	*T*	*AA*	*AT*	*TT*	*Ho*	*He*
	0.04	0.96	0.00	0.08	0.92	0.076	0.077
MSTN/T*aqI*	C	T	CC	CT	TT		
	1.00	0	1.00	0.00	0.00	-	-
RYR1	N	n	NN	Nn	nn		
	0.98	0.02	0.96	0.08	0.00	0.038	0.039

Bašić et al. (2012) studied 873 individuals from six commercial pig breeds. They reported the lowest number of heterozygous carriers of PSS mutation in Hampshire swine, and the highest was in Hypor (commercial synthetic line). Out of the common breeds Landrace swine were affected much higher than Yorkshire and the crossbreed breeds. The authors concluded that in general the data showed that absence of both recessive genotypes (nn) and heterozygotes (Nn) genotype of RYRI gene among breeds. The molecular method described here offers a new tool for quick and inexpen-sive detection of all carriers of mutated RYRI gene in pigs.

Davalos-Aranda et al. (2005) announced similar results. They studied some commercial breeds and determined low frequency of the PSS mutation in higher muscular porcine breeds, such as Pietrain and Large White.

In previous our study (Stoykova-Grigorova et al., 2015) were genotyped 53 animals from Dunavska white breed for RYR1 gene and two genotypes were established – homozygous genotype NN with frequency 0.962 and heterozygous genotype Nn – 0.037. The presence of recessive allele could be result from participation of breeds Pietrain and Landrace in the process of breeding. In those two breeds Tăbăran (2000), Russo et al. (2004) and Cieślak et al. (2003) reported high frequencies of mutation in MSTN and RYR1 loci.

The present investigation was one of the few performed in Bulgaria. It gave important information about genetic resources in pig breeding and this data could be implemented in management of breeding programs.

Conclusion

The PCR-RFLP method was suitable for detection of polymorphism in pig genes. After restriction analysis of two regions in MSTN pig gene with two specific endonucleases the results revealed allele diversity only in the promotor region. The analysis showed that exon 3 was monomorphic in the investigated population. Two alleles of the RYR1 gene were identified in the studied population. The majority of the animals were homozygous by the dominant *NN* alleles related to high quality meat.

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