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Full Length Research Paper

The impact of *MYOG*, *MYF6* and *MYOD1* genes on meat quality traits in crossbred pigs

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The objective of this paper was to determine the effect of the *MYOG*, *MYF6* and *MYOD1* genes on selected meat quality traits in crossbred pigs. The observation of these effects on the total amount of 20 indicators of meat quality traits was carried out on a sample of 124 slaughter pigs of cross-breed combinations Pietrain × (Large White$_D$ × Landrace) and (Large White$_S$ × Duroc) × (Large White$_D$ × Landrace), all tested at the Test and Experiment Station of the Czech University of Life Sciences, Prague. The *MYOG* gene was discovered to have a significant effect on the water content in the ham and shoulder and on the intramuscular fat content in the shoulder. Concerning the MYF4 locus, the pigs of the AA genotype showed a higher content of intramuscular fat (P < 0.01) than the pigs of the BB genotype. The *MYOD1* gene had a statistically significant effect on the water content in the ham and loin and on the muscle area of the belly (the belly fat share). Also, regarding the MYOD1 locus, the pigs of the AA genotype showed a higher lean meat content in the belly (P < 0.01) than the pigs of the BB genotype. Based on the results of this study, it can be stated that mutations in the MYOG, MYF6 and MYOD1 genes show a significant effect on the pork meat quality.

Key words: Pig, meat quality, MyoD family genes.

INTRODUCTION

In the last decade, there has been a notable progress observed in the area of the performance of a number of economically important traits characterising farm animals. This progress has been enabled by the connection of molecular genetics with artificial selection and introgression (Dekkers and Hospital, 2002). Despite the fact that most of these traits belong among quantitative traits, controlled by polygenes and factors of the outer environment, there was a high genetical gain achieved. This stands true even though the observable phenotypic qualities (breeding value of an individual) are a result of a complicated complex containing a number of factors and genes (Georges, 2007). There is a significant number of genes (Davoli et al., 2003; Fontanesi et al., 2011; Srikanchai et al., 2010; Rotschild et al., 2010; Cinar et al., 2012) and quantitative trait loci (QTL) (www.animalgenome.org/cgi-bin/QTLdb/SS) that have been reported for meat and carcass quality traits in pig. The process of the origin and development of the vertebrate muscle tissue is controlled by a large number of regulatory pathways and genes. The activation of embryonic cells taking part in the process of myogenesis is controlled by series of complex regulatory transcriptional pathways, the result of which being the expression of so called myogenic regulatory factors (MRF) (Fan et al., 2011). The MRF gene family is composed of four structurally related transcription factors – *MYOG*, *MYF6*, *MYF5* and *MYOD1* – regulating both the development of skeletal muscle fibers and postnatal growth hypertrophy (Te Pas et al., 2004; Cinar and Fan, 2012). The MRF genes are therefore considered potential candidate genes for investigation of mutual interactions and their impact on the development of skeletal muscle tissue (Ujan et al., 2011).

Each MRF family gene expression has a specific impact on the process of myogenesis. The expression of the *MYF5* and *MYOD1* (*MYF3*) genes plays a fundamental role during myoblast proliferation, while the

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Table 1. Genotype frequencies for the investigated genes.

Parameter	MYOG/MspI			MYF6/BseRI			MYOD1/DdeI		
	AA	AB	BB	AA	AB	BB	AA	AB	BB
n	70	43	8	22	66	25	32	70	22
%	57.85	35.54	6.61	19.47	58.41	22.12	25.81	56.45	17.74

expression of the *MYOG* (*MYF4*, myogenin) and the *MYF6* (*MRF4*) genes is linked to the differentiation and maturation of myofibers (Wyszynska-Koko and Kuryl, 2004). Myogenin (*MYOG*) plays a key role in the differentiation of muscles, controls the onset of myoblast fusion and formation of myofibers (Soumillion et al., 1997). The MYF6 gene is expressed postnatally and shows ten times the efficiency of the other *MYOD* family genes (Bober et al., 1991). The *MYOD1* gene, together with the *MYF5* gene, participates in the process of myoblast proliferation (Klosowska et al., 2004).

The aim of this paper is to determine the effect of the MYOG (X89209.1:g.174G>C), MYF6 (DQ139775.1:g.1229c>g) and MYOD1 (U12574:g.1264C>A) genes on qualitative carcass traits value (pH, electrical conductivity, meat colour, tenderness, drip loss, belly tissue composition, water and intramuscular fat content in the ham, neck, loin and shoulder) of crossbred pigs.

MATERIALS AND METHODS

During the course of monitoring the effects of *MYOG*, *MYF6* and *MYOD1*genes on the meat quality traits, there were 124 pigs of the crossbred combination Pn × (LW_D × L) (62 pigs) and (LW_S × D) × (LW_D × L) (62 pigs) tested at the Testing and Experimental Station of the Czech University of Life Sciences, Prague. The pigs were fed with the commercial feeding mixtures divided into 3 separate phases with continual transfer in between them. After reaching a live weight of about 123 kg at age 170 days, the animals were slaughtered at a commercial slaughterhouse. The carcasses were dissected using the method of Walstra and Merkus (1996). The belly was cut into two parts between 10. and 11. rib. The resulting cuts was then analysed with the use of image analyses, observing the following parameters: muscle area (mm^2), total area of the section (mm^2) and the ratio of the muscle area (%).

For the needs of this experiment the following indicators were monitored: pH value and electrical conductivity – 45, resp. 50 min *post mortem* in the *musculus longissimus* (pH ML 45, electrical conductivity of ML 50) and *musculus semimembranosus* (pH MS 45, electrical conductivity of MS), meat colour – with the use of a spectrophotometer Minolta CM 700, drip loss (%), meat tenderness (kg), intramuscular fat content of the main meat parts (%) determined by the Soxhlet method (using a gravimetric determination) and the water content in the main meat parts (%), again using a gravimetric determination (AOAC, 1990).

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) gene polymorphism was determined with the use of the following methods: 3' flanking region of the *MYOG*/*Msp*I (X89209.1:g.174G>C) gene as per Soumillion et al. (1997), intron 1 of the *MYF6*/*Bse*RI gene (DQ139775.1:g.1229c>g) (*Bse*RI) as per Vykoukalova et al. (2003), *MYOD1*/*Dde*I gene (U12574:g.1264C>A) (*Dde*I) as per Knoll et al. (1997).

The effect of a missense mutation of the *MYOG, MYF6* and *MYOD1* gene on the qualitative traits was analysed using the UNIVARIATE, MEANS and GLM (type IV) procedures (SAS®, Institute, 2001). The model included the *MYOG, MYF6* and *MYOD1* genotype, crossbred combination and gender as fixed factors and the carcass weight as a regression coefficient. The formula used for obtaining the results is:

$$Y_{ijklmn} = \mu + G_i + F_j + D_k + S_l + C_m + \beta x_n + e_{ijklmn}$$

Where, Y_{ijklmn} = the value of the trait; μ = the overall mean; G_i = the effect of *MYOG* genotype (i = 1, 2, 3); F_j = the effect of *MYF6* genotype (j = 1, 2, 3); D_k = the effect of *MYOD1* genotype (k = 1, 2, 3); S_l = the effect of sex (l = 1, 2); C_m = the effect of crossbred combination (m = 1, 2); β = the regression coefficient of carcass weight; e_{ijklmn} = the random residual error.

RESULTS AND DISCUSSION

For the study sample, the genotypes of three different genes (*MYOG*, *MYF6*, *MYOD1*) were determined. Three genotypes were established for each gene. The frequency of individual genotypes, for both sexes and all crossbred combinations, are listed in Table 1. The most frequent genotype of the *MYOG* gene was the *AA* genotype, while the *BB* genotype was the least frequent. The most frequent genotype for both the *MYF6* and *MYOD1* genes was the *AB* genotype (Table 1).

The effect of the *MYOD* group of genes on the meat quality traits in pigs has been investigated by several different authors (Cieslak et al. 2002; Krzecio et al. 2007; Kapelanski et al. 2005; Cinar et al. 2012). The mechanistic basis for the functional redundancy of the MYOD group in myotome formation has been recently examined by lineage-ablation experiments in the mouse (Bryson-Richardson and Currie, 2008).

For the *MYOG* gene (Table 2), a statistically significant effect was found concerning the water content in the ham and shoulder and the intramuscular fat content in the shoulder. Kapelanski et al. (2005) reported statistically significant differences between the *MYOG* genotypes for colours L*, a* and for drip loss. In agreement with our results, the afore-cited authors found no correlation between the gene and the pH value, water content in the loin and intramuscular fat content in the loin. Moreover, the *MYOD1* gene was found to have an important effect on the water content in the ham, loin and on the muscle area of the belly.

Table 2. The effect of the investigated genes and their interactions on meat quality indicators and chemical analysis indicators.

Trait	MYOG	MYF6	MYOD1	Cross-bred	Sex	P - value
	Significance	Significance	Significance	Significance	Significance	
	F emp.=	F emp.=	F emp.=	F emp.=	F emp.=	
pH MS 45	0.76 (NS)	0.09 (NS)	0.59 (NS)	0.77 (NS)	0.01 (NS)	0.4356
pH ML 45	0.22 (NS)	0.12 (NS)	0.21 (NS)	0.71 (NS)	0.54 (NS)	0.8939
Electrical conductivity MS	0.79 (NS)	0.67 (NS)	1.49 (NS)	12.21**	0.36 (NS)	0.0006
Electrical conductivity ML	0.40 (NS)	0.06 (NS)	0.02 (NS)	14.46**	0.01 (NS)	0.0010
Meat colour L	0.12 (NS)	0.65 (NS)	0.12 (NS)	.	0.44 (NS)	0.4944
Meat colour a	1.03 (NS)	1.44 (NS)	1.82 (NS)	.	2.10 (NS)	0.3858
Meat colour b	0 (NS)	0.57 (NS)	1.32 (NS)	.	3.87 (NS)	0.4518
Meat tenderness (kg)	0.78 (NS)	0.36 (NS)	0.45 (NS)	0.39 (NS)	0.04 (NS)	0.0552
Drip loss (%)	0.39 (NS)	0.04 (NS)	0.16 (NS)	13.45**	0.02 (NS)	0.0015
Area of meat of part 2 of the belly (mm^2)	0.04 (NS)	0.54 (NS)	2.71 (NS)	1.06 (NS)	0.47 (NS)	0.7582
Total area of part 2 of the belly (mm^2)	0.64 (NS)	1.07 (NS)	0.17 (NS)	1.40 (NS)	2.64 (NS)	0.6046
Ratio of area of meat of part 2 of the belly (%)	1.61 (NS)	0.84 (NS)	4.50*	0.65 (NS)	11.76**	0.0003
Water content in the ham (%)	7.52**	0.98 (NS)	4.03*	.	0.50 (NS)	0.0613
Water content in the neck (%)	0.76 (NS)	0.56 (NS)	0.81 (NS)	.	0.80 (NS)	0.2544
Water content in the loin (%)	3.04 (NS)	1.55 (NS)	5.31*	.	6.54*	0.0751
Water content in the shoulder (%)	3.73*	1.96 (NS)	0.60 (NS)	.	1.46 (NS)	0.4108
Intramuscular fat content for the ham (%)	1.70 (NS)	0.12 (NS)	2.87 (NS)	.	0.59 (NS)	0.3822
Intramuscular fat content for the neck (%)	0.02 (NS)	0.24 (NS)	0.52 (NS)	.	1.27 (NS)	0.7907
Intramuscular fat content for the loin (%)	0.78 (NS)	0.89 (NS)	0.40 (NS)	.	1.28 (NS)	0.7328
Intramuscular fat content for the shoulder (%)	5.1*	2.58 (NS)	0.45 (NS)	.	3 (NS)	0.2866

NS – Statistically not significant. * statistically significant at P ≤ 0.05. ** statistically significant at P ≤ 0.01.

Table 3. Least square mean of investigated indicators for the MYOG genotypes.

Trait	Genotype		
	AA	AB	BB
Water content in the ham (%)	74.28 ± 0.81	75.69 ± 1.45^{A}	73.11 ± 0.75^{B}
Water content in the shoulder (%)	73.74 ± 0.27^{a}	74.11 ± 0.38	74.78 ± 0.91^{a}
Intramuscular fat content for the shoulder (%)	3.59 ± 0.29^{A}	2.73 ± 0.28^{Ba}	2.62 ± 0.37^{b}

a, b – P<0.05. A, B – P < 0.01.

Verner et al. (2007) reported a statistically significant effect of the *MYOD1* gene on the dry matter content of the loin. Kuryl et al. (2002) found no statistically significant differences between the effects of the *MYOG* genotypes on the selected yield indicators. Contrary to this finding, Kapelanski et al. (2005) found a statistically significant effect of the *MYOG* genomes on drip loss and loss by boiling (as well as on colour) and a significant effect of the *MYF6* genotypes on drip loss. Verner et al. (2007) mentioned statistically significant differences between *MYOG* genotypes regarding their effect on intramuscular fat, neck weight, loin weight and muscle lean meat share. No statistically significant differences were found between the *MYF6* genotypes. For the *MYOD1* gene, statistically significant differences were determined for the intramuscular fat content and dry matter content indicators.

Animals of the *BB* genotype (carrying the *MYOG* gene) showed lower weights of the fat and skin cover of the shoulder (data not shown), the highest proportion of loin in dressed carcass and the highest weight of the loin without fat and skin cover. Animals with this genotype also demonstrated lower intramuscular fat content in the shoulder (Table 3). Due to the higher water content in the muscle (compared to adipose tissue) animals of the *BB* genotype showed the highest water content in the shoulder. This finding applied only to the shoulder area, which can be explained by the higher share of the intramuscular fat (3.59 to 2.62%) in the shoulder than in the loin. Kapelanski et al. (2005) states, that the amount of intramuscular fat in the loin equals 1.86%. Higher fat content is related to a lower content of water, which is

Table 4. Least square mean of investigated indicators for the MYOD1 genotypes.

Trait	Genotype		
	AA	AB	BB
Ratio of area of meat of part 2 of the belly (%)	63.32 ± 1.51^{a}	64.44 ± 1.66^{A}	61.62 ± 1.89^{Ab}
Water content in the ham (%)	72.85 ± 1.41^{A}	76.08 ± 1.48^{B}	75.31 ± 2.21
Water content in the loin (%)	72.13 ± 0.40^{a}	73.45 ± 0.42^{b}	73.09 ± 0.63

a, b – P ≤ 0.05. A, B – P ≤ 0.01.

why the loin of the *AA* and *AB* genotype pigs shows higher water content, in accordance with the work of Kapelanski et al. (2005).

Concerning the *MYOD1* gene (Table 4), the lowest water content in the ham and loin was found in animals of the *AA* genotype (data not shown). The association of the c.746G>A missense mutation with fatness and growth rate has been confirmed in several populations of pigs with a different genetic background (Kim et al., 2000; Houston et al., 2004; Van den Maagdenberg et al., 2007; Fan et al., 2011). However, some studies did not detect significant effects of this mutation on production traits. These varying results were probably caused by differences in the genetic background. Cinar et al. (2012) described the regulatory mechanism of MYF6 and MYF5 and the expression of both genes, which is in part activated together and differentially mRNA regulation of MYF6 between Duroc and Pietrain pigs was shown previously. Our study used 2 crossbred sire lines - Pietrain and Sire Large White with Duroc. The effect of breed was notable in the case of electrical conductivity and drip loss indicators. However, none of the monitored genes showed any interaction between the cross-breed and indicators of meat quality.

The significant effect of genotypes at MYOG and MYOD1 loci on meat traits was observed especially in the traits connected to glycolysis and contractile properties of the cytoskeletal proteins (such as pH, drip loss, pale colour). Based on the obtained results, it can be said that mutations in the coding and non-coding regions of MyoD genes show significant effects on a variety of muscle characteristics and thereby on the quality of the meat.

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REFERENCES

AOAC (Association of Official Analytical Chemists) (1990). Official Methods of Analysis 15th ed. 1990.

Bober E, Lyons GE, Braun T, Cossu G, Buckingham M, Arnold HH (1991). The muscle regulatory gene, MYF-6, has a biphasic pattern of expression during early mouse development. J. Cell Biol. 113(6):1255-1265.

Bryson-Richardson RJ, Currie PD (2008). The genetics of vertebrate myogenesis. Nat. Rev. Genet. 9(8):632-646.

Cieslak D, Kuryl J, Kapelanski W, Pierzchala M, Grajewska S, Bocian M (2002). A relationship between genotypes at MYOG, MYF3 and MYF5 loci and carcass meat and fat deposition traits in pigs. Anim. Sci. Pap. Rep. 20(2):77-92.

Cinar MU, Fan HT (2012b). The mRNA expression pattern of skeletal muscle regulatory factors in divergent phenotype swine breeds. Kafkas Univ. Vet. Fak. Derg. 18(4):685-690.

Cinar MU, Fan HT, Neuhoff C, Grobe-Brinkhaus C (2012). eQTL Analysis and association of MYF6 mRNA expression with meat quality traits in pigs. Kafkas Univ. Vet. Fak. Derg. 18(2):235-242.

Davoli R, Fontanesi L, Cagnazzo M, Scotti E, Buttazzoni L, Yerle M, Russo V (2003). Identification of SNPs, mapping and analysis of allele frequencies in two candidate genes for meat production traits: The porcine myosin heavy chain 2B (MYH4) and the skeletal muscle myosin regulatory light chain 2 (HUMMLC2B). Anim. Genet. 34(3):221-225.

Dekkers JCM, Hospital F (2002). The use of molecular genetics in improvement of agricultural populations. Nat. Rev. Genet. 3(1):22-32.

Fan HT, Cinar MU, Mehmet U, Phatsara C, Tholen E, Looft C, Schellander K (2011). Molecular mechanism underlying the differential MYF6 expression in postnatal skeletal muscle of Duroc and Pietrain breeds. Gene 486(1-2):8-14.

Fontanesi L, Colombo M, Tognazzi L, Scotti E, Buttazzoni L, Dall'Olio S, Davoli R, Russo V (2011). The porcine TBC1D1 gene: Mapping, SNP identification, and association study with meat, carcass and production traits in Italian heavy pigs. Mol. Biol. Rep. 38(2):1425-1431.

Georges M (2007). Mapping, fine mapping, and molecular dissection of quantitative trait loci in domestic animals. Annu. Rev. Genomics Hum. Genet. 8:131-162.

Houston RD, Cameron ND, Rance KA (2004). A melanocortin-4 receptor (MC4R) polymorphism is associated with performance traits in divergently selected large white pig populations. Anim. Genet. 35(5):386-390.

Kapelanski W, Grajewska S, Kuryl J, Bocian M, Wyszynska-Koko J, Urbanski P (2005). Polymorphism in coding and non-coding regions of the MyoD gene family and meat quality in pigs. Folia Biol. Krakow. 53(S):45-49.

Kim KS, Larsen N, Short T, Plastow G, Rothschild M.F. (2000): A missense variant of the porcine melanocortin-4 receptor (MC4R) gene is associated with fatness, growth, and feed intake traits. Mamm. Genome 11(2):131-135.

Klosowska D, Kuryl J, Elminowska-Wenda G, Kapelanski W, Walasik K, Pierzchala M, Cieslak D, Bogucka J (2004). A relationship between the PCR-RFLP polymorphism in porcine MYOG, MYOD1 and MYF5 genes and microstructural characteristics of *M. longissimus lumborum* in Pietrain × (Polish Large White × Polish Landrace) crosses. Czech J. Anim. Sci. 49(3):99-107.

Knoll A, Nebola M, Dvorak J, Cepica S (1997). Detection of a DdeI PCR RFLP within intron 1 of the porcine MYOD1 (MYF3) locus. Anim. Genet. 28(4):308-322.

Krzecio E, Kocwin-Podsiasla M, Kuryl J, Zybert A, Sieczkowska H, Antosik K (2007). The effect of genotypes at loci CAST/MspI (calpastatin) and MYOG (myogenin) and their interaction on selected productive traits of porkers free of gene RYR1T. I. Muscling and

morphological composition of carcass. Anim. Sci. Pap. Rep. 25(1): 5-16.
Kuryl J, Kapelanski W, Cieslak D, Pierzchala M, Grajewska S, Bocian M (2002). Are polymorphisms in non-coding regions of porcine MyoD genes suitable for predicting meat and fat deposition in the carcass? Anim. Sci. Pap. Rep. 20(4): 245-254.
Rotschild MF, Fan B, Lkhagvadorj S, Cai W, Young J, Smith RM, Dekkers JCM, Huff-Lonergan E, Lonergan SM (2010). Identification of genetic markers associated with residual feed intake and meat quality traits in the pig. Meat Sci. 84(4):645-650.
SAS® Propriety Software Release 9.1, of the SAS® system for Microsoft® Windows®. SAS Institute Inc., Cary, NC., 2001.
Soumillion A, Erkens JHF, Lenstra JA, Rettenberger G, tePas MFW (1997). Genetic variation in the porcine myogenin gene locus. Mamm. Genome 8(8):564-568.
Srikanchai T, Murani E, Phatsara C, Schwerin M, Schellander K, Wimmers K, Ponsuksili S (2010). Association of ZYX polymorphisms with carcass and meat quality traits in commercial pigs. Meat Sci. 84(1):159-164.
Te Pas MFW, Everts ME, Haagsman HP (2004). Muscle development of livestock animals: physiology, genetics and meat quality. CABI Publishing, Wallingford, Oxfordshire, UK. p. 411.
Ujan JA, Zan LS, Shengjuan W, Adoligbe C, Wang HB (2011). Meat tenderness and water holding capacity are associated with a 959 A G mutation in the MyoG gene of Chinese indigenous cattle. Afr. J. Biotechnol. 10(29):5654-5660.
Van den MK, Stinckens A, Claeys E, Seynaeve M, Clinquart A, Georges M, Buys N, De Smet S (2007). The Asp298Asn missense mutation in the porcine melanocortin-4 receptor (MC4R) gene can be used to affect growth and carcass traits without an effect on meat quality. Animal 1(8):1089-1098.
Verner J, Humpolicek P, Knoll A (2007). Impact of MYOD family genes on pork traits in Large White and Landrace pigs. J. Anim. Breed. Genet. 124(2):81-85.
Vykoukalova Z, Knoll A, Dvorak J, Rohrer G A, Cepica S (2003). Linkage and radiation hybrid mapping of the porcine MYF6 gene to chromosome 5. Anim. Genet. 34(3):238-240.
Walstra P, Merkus GSM (1996). Procedure for assessment of the lean meat percentage as a consequence of the new EU reference dissection method in pig carcass classification. DLO- Research Institute for Animal Science and Health Research Branch. Zeist, The Netherlands pp. 1-22.
Wyszynska-Koko J, Kuryl J (2004). Porcine MYF6 gene: sequence, homology analysis, and variation in the promoter region. Anim. Biotechnol. 15(2):159-173.