Praca oryginalna ***Original paper***

Slaughter value and meat quality of fattening pigs in relation to genotype at the *MYOG* locus

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Received 17.09.2024 *Accepted 10.12.2024*

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Summary

The aim of the study was to determine the quality of carcasses, technological and consumption suitability of raw slaughter material in relation to crossbred fatteners of selected breeds of pigs with known genotype at the *MYOG* locus. The experiment used 90 crossbred fattening pigs of the breeds Polish Large White (PLW), Polish Landrace (PL), Duroc, Pietrain, with known genotype at the *MYOG* locus. The polymorphism of the *MYOG* gene was determined by the PCR-RLFP method, using the restriction enzyme MspI. Relationships between individual *MYOG* genotypes and carcass lean and fat content, as well as physical characteristics and chemical composition of loin and ham were determined. It was found that fatteners with the AA genotype had the highest loin eye height and the highest weight of whole ham and ham without skin. It was also noted that the loins and hams of fatteners with the AA genotype contained the highest amount of free water. In contrast, loins with significantly higher fat content compared to the other genotypes were obtained from fattening pigs with genotype BB, while at the same time proving to be the most caloric.

Keywords: pigs, *MYOG* gene, slaughter value, meat quality

The quality traits of pork carcasses and meat are determined by environmental and genetic factors, among which, until recently, breed was mentioned above all. In view of developments in the science of molecular genetics, the leading role of genetic markers as factors determining the value of phenotypic traits is increasingly being pointed out. Among these, the myogenin gene (*MYOG*), which belongs to the *MyoD* family responsible for myogenesis, is mentioned (5). As stated, muscle fibre formation takes place during the embryonic phase of development and is regulated by genes from the *MyoD* family. It includes 4 genes with similar structures: *MYOG* or *MYF4* (myogenin), *MYOD1* (myogenic determination factor), *MYF5* (myogenic factor 5), *MYF6* or *MRF4* (myogenic factor 6) (7). The myogenin gene (*MYOG*) is one of the main genetic factors that control the differentiation and maturation of muscle fibres (21). Therefore, it has been recognised as an important genetic marker for slaughter traits in pigs. The *MYOG* gene showed three polymorphic sites identified by the restriction enzyme MspI: in the promoter region, the second intron and the 3'region of the gene (20). Kurył et al. (13), on the basis of their study, showed that the *MYF4* (*MYOG*) genotype had a significant effect on ham weight and meat content, loin eye area and proportion of lean meat in the carcass. In contrast, a study by Kłosowska et al. (11) showed no significant association of genotypes of selected genes from the *MyoD* family with meat quality apart from the effect of alleles of the studied genes on microstructure traits of the *longissimus dorsi* muscle.

The inconclusiveness of the above research results indicates the relevance of undertaking further studies, especially those carried out on the national pig population, which shows a good quality of slaughter material for the production of culinary meat and high-quality cured meats (1).

The aim of the study was to determine the quality of pork carcasses and the physical and chemical parameters of the meat in relation to the polymorphism at the *MYOG* locus.

Material and methods

The analysis included crossbred fattening pigs (F1♀ (♀Polish Large White × ♂Polish Landrace) × F1♂ (♀Duroc × ♂Pietrain)) kept in an individual farm on litter according to welfare requirements (14). The farm used a dry system feeding *ad libitum* with a mixture containing 5.8% crude protein and 13 MJ ME/kg feed in the first phase of fattening, i.e. up to 70 kg body weight, and 14.2% protein and 12.5 MJ ME/kg feed in the second phase, i.e. from 70 kg to the end of fattening.

The fattening pigs were slaughtered in the Meat Processing Plant at a body weight of 117 kg (± 2 kg). Carcass meat percentage was determined using the SYDEL CGM apparatus, code: YXX20123.01.T01. Measurements were taken using the loin method by determining the thickness of the backfat and loin eye height at point C_7, i.e. at the height of the last rib at a distance of 7 cm from the splitting line of the carcass. After a 24-hour cooling at 2-4°C, the right half-carcasses were dissected into the main cuts, including those intended for further analysis: the loin and the ham. These cuts were dissected taking into account their weight: without skin, without skin and bone, without skin, bone and fat. Samples of 200 g for laboratory tests were taken from loin (*musculus longissimus dorsi*) and ham (*musculus semimembranosus*) to determine physical and chemical characteristics. The pH of the loin and ham were measured 45 minutes and 24 hours after slaughtering with a Matthäus PH-Star CPU apparatus. The percentage of free water (water-holding capacity – WHC) was determined using the method of Grau and Hamm (1952) (6) as modified by Pohja and Niinivaara (1957) (17). Using the FoodScan™ meat analyser (FOSS), which uses near-infrared (NIR) transmission spectrometry, the percentage of water, total protein, total fat and collagen were determined according to PN-A-82109 (2010) (16). The gross caloric value (kJ 100 g^{-1}) was calculated on the basis of the content of total protein and fat, assuming energy equivalents for 1 g of protein and fat: 17 kJ and 37 kJ, respectively (18).

After previously performed genotype testing, a group of 90 fattening pigs (gilts), 30 in each genotype of the *MYOG* gene (AA, AB, BB), was allocated for analysis.

The biological material for DNA testing consisted of samples of loin muscle tissue. DNA isolation was performed using Qiagen's ready-to-use kits: the DNeasy® Blood &Tissue Kit, according to the procedure provided by the manufacturer. The PCR reaction was performed using a PTC-200 Peltier Thermal Cycler (MJ Research). Primer sequences for the myogenin (*MYOG*) gene were prepared from literature data (20). Synthesis of primer sequences was performed at Genomed Ltd. and Sigma-Aldrich Ltd. The total volume of the PCR reaction mixture for each sample was 15 µl and contained 7.5 µl of REDTaq® ReadyMix™ PCR Reaction Mix reagent (Sigma-Aldrich), 0.2 µl (5 µM) of each primer (Sigma-Aldrich/Genomed), 5.9 µl of nuclease-free water (Sigma-Aldrich) and 1.2 µl (20 ng/µl) of DNA. Thermal conditions (Tab. 1) were refined with PCR test reactions in a temperature gradient. The restriction enzyme MspI from BioLabs Inc. was used to digest the obtained PCR products. Electrophoretic separation of the obtained PCR product, after restriction enzyme digestion, was performed in agarose gel (SIGMA).

Tab. 1. PCR reaction conditions for the *MYOG* gene

Position in the chromosome	Allele	Annealing temperature	Primer sequences (5'→ 3')
9q2.1-q2.6	A – 353 B – 219,134	60°C	F 5'-TCAGGAAGAACTGAAGGCTG-3' R 5'-GTTTCCTGGGGTGTTGC-3'

Statistical analysis was performed with SAS software (version 9.4 by SAS Institute Inc. Cary, NC) using multivariate analysis of variance. Verification of the values of the analysed traits was performed using a mixed model:

$$y_{ij} = \mu + G_i + b + e_{ij}$$

which included:
y_{ij} – the value of the studied trait,
μ – the mean value of the trait,
G_i – genotype fixed effect at the locus under study,
b – regression coefficient for slaughter age,
e_{ij} – random error.

Results and discussion

Carcass quality indicators. Table 2 shows the carcass muscling and fatness characteristics of fattening pigs with a specific genotype at the *MYOG* locus. A statistically significant association was found between the polymorphism at the *MYOG* locus and loin cross-sectional height. The reported differences between AA > AB genotypes averaged 4.1 mm. However, the polymorphism of this gene had no effect on the carcass meat percentage of the fatteners, which is in line with the results of studies by Kłosowska et al. (11), as well as Rybarczyk et al. (19). The opposite results were obtained in the study of Čechová and Mikule (4), where it was found that to achieve high carcass muscling indices, the *MYOG* BB genotype was the most favourable (greater loin muscle area and higher carcass meat percentage). In the case of backfat thickness, our study reported that the thickest backfat was characteristic of heterozygotes (AB), but these differences were found to be statistically insignificant, as was also found in studies by Kłosowska et al. (11), Čechová and Mikule (4) and Rybarczyk et al. (19). The differences observed above in the effect of the

Tab. 2. Carcass muscling and fatness characteristics of crossbred fatteners depending on the genotype of the *MYOG* gene

MYOG genotype		Backfat thickness (mm)	Loin eye height (mm)	Meatiness (%)
AA	$\bar{x}$	16.60	68.2[a]	57.9
	SD	4.27	3.48	2.3
BB	$\bar{x}$	17.18	66.9[ab]	57.4
	SD	4.58	5.19	2.9
AB	$\bar{x}$	17.40	64.1[b]	57.0
	SD	2.03	3.47	2.01

Explanations: a, b – values in columns with different letters differ significantly ($P \leq 0.05$)

Tab. 3. Loin and ham dissection results in relation to fattening pig genotype at the *MYOG* locus

MYOG genotype		Whole loin (kg)	Loin without skin (kg)	Loin without skin and bone (kg)	Loin without skin, bone and fat (kg)	Whole ham (kg)	Ham without skin (kg)	Ham without skin and bone (kg)	Ham without skin, bone and fat (kg)
AA	$\bar{x}$	6.79	5.40	5.12	4.28	13.21^{a}	12.26^{b}	11.23	9.81
	SD	0.73	0.54	0.52	0.32	0.94	0.79	0.66	0.68
BB	$\bar{x}$	6.62	5.31	5.03	4.23	13.12^{ab}	12.10^{b}	11.31	9.87
	SD	0.79	0.58	0.35	0.40	0.99	0.68	0.54	0.82
AB	$\bar{x}$	6.01	5.18	4.57	3.72	12.34^{b}	11.29^{a}	10.49	9.14
	SD	0.59	0.46	0.36	0.30	0.74	0.58	0.41	0.62

Explanations: a, b – differences statistically significant at $P \leq 0.05$

MYOG gene on carcass lean content may be due to the use of different animal material for the study. In our study, four-breed hybrids were used with infusion of the Pietrain and Duroc paternal breeds, while in the study by Čechová and Mikule (4), the Czech Landrace and Czech Large White breeds were used, although it should be emphasised that both Czech and Polish breeds belong to the same meat type of pigs.

Results of loin and ham dissection. One of the methods used to determine the proportion of individual tissues in the carcass is the dissection of the most valuable carcass cuts, i.e. loin and ham (22). At the same time, it is an important indicator for determining the commercial value of the carcass and thus the profitability of producing crossbred fatteners obtained from a given crossbreeding variant (12). As reported by Tyra (22), in purebred PLW, PL, Duroc and Pietrain individuals evaluated in 2022 at the Pig Performance Testing Station, rump ham weight without fat and skin was 10.29 kg, 10.36 kg, 10.40 kg and 11.25 kg, respectively. The ham values without skin obtained in our study for the hybrids of these breeds averaged 11.88 kg, which is a similar value for the parental breeds.

Analysing the association of the polymorphism at the *MYOG* locus with loin and ham weight (Tab. 3), it was found that individuals with the AA genotype of this gene were only characterised by statistically significantly higher whole ham weight as well as ham weight without skin ($P \leq 0.05$). The recorded differences between AA > AB genotypes for whole ham averaged 0.87 kg. For ham without skin, individuals with AA and BB genotypes had a heavier ham than AB individuals by 0.97 kg and 0.81 kg, respectively.

Physical characteristics of loin and ham. Tables 4 and 5 show the pH values and water holding capacity for loin and ham. Analysis of the relationship between the polymorphism at the *MYOG* locus and the pH value showed no statistically significant differences between the analysed genotypes. The lack of effect of this polymorphism on the pH of pork loin was also shown by Rybarczyk et al. (19). The pH values obtained were comparable to those characteristic of the domestic PL breed, i.e. pH_{45} 6.16-6.28 and pH_{24} 5.62-5.64 (15). Also Choi et al. (3), who conducted a study on Landrace × Yorkshire × Duroc crossbreds, showed a similar pH in the loin after 24 h carcass chilling of 5.58. Lower values were obtained by Kaić et al. (8) who examined the loin of Pietrain crossbreds with Swedish Landrace × Large White and German Landrace × Large White breeds. These authors reported pH_{45} and pH_{24} values of 6.08 and 5.94 and 5.95 and 5.46, respectively, which may be the result of using only breeds with outstanding lean meat percentage.

Tab. 4. Physical properties of loin in relation to fattening pig genotype at the *MYOG* locus

MYOG genotype		pH_{45}	pH_{24}	WHC
AA	$\bar{x}$	6.43	5.65	25.07^{a}
	SD	0.30	0.19	6.14
BB	$\bar{x}$	6.30	5.61	23.39^{b}
	SD	0.31	0.04	2.74
AB	$\bar{x}$	6.34	5.62	24.01^{ab}
	SD	0.27	0.17	4.67

Explanations: a, b – differences statistically significant at $P \leq 0.05$

Tab. 5. Physical properties of ham in relation to fattening pig genotype at the *MYOG* locus

MYOG genotype		pH_{45}	pH_{24}	WHC
AA	$\bar{x}$	6.34	5.69	16.58^{a}
	SD	0.18	0.30	2.62
BB	$\bar{x}$	6.37	5.74	14.45^{b}
	SD	0.33	0.24	1.89
AB	$\bar{x}$	6.49	5.72	15.31^{ab}
	SD	0.32	0.27	1.38

Explanations: a, b – differences statistically significant at $P \leq 0.05$

Kłosowska et al. (11) demonstrated the influence of genes from the *MyoD* family, including myogenin, on loin muscle microstructure traits in Pietrain × (PLW × PL) hybrids, which is important for meat quality traits in particular, including water holding capacity. The WHC parameter, calculated in the experiment as a percentage of free water, is an important source of information on meat quality. The higher it is, the more watery the meat is. For this parameter, the polymorphism of the myogenin gene was found to have

a statistically significant effect on its value in both loin and ham of fattening pigs. In loin and ham, positively the lowest WHC value was recorded in BB homozygous individuals. The magnitude of this parameter was significantly lower than in individuals with the AA genotype (BB < AA, $P \leq 0.05$) by 1.68% in loin and by 2.13% in ham. Similar to Rybarczyk et al. (19) no differences were found in WHC values between individuals with AA and AB genotypes. Cebulska et al. (2), analysing the WHC of meat from (PLW × PL) × Duroc crossbreds, showed a value of 17.82%. On the other hand, the percentage of free water in the loin of PL fatteners, according to different studies, ranged from 25.29 (9) to 35.90 (23).

Tab. 6. Chemical composition and energy value of loin in relation to fattening pig genotype at the *MYOG* locus

MYOG genotype		Water (%)	Fat (%)	Protein (%)	Collagen (%)	Energy value (kJ/100 g)
AA	$\bar{x}$	73.45	2.01[b]	23.02	1.01	465.71
	SD	6.72	0.64	2.15	0.09	38.74
AB	$\bar{x}$	72.87	1.95[b]	23.69	1.04	474.88[ab]
	SD	6.95	0.77	2.66	0.10	52.67
BB	$\bar{x}$	72.06	2.71[a]	23.43	0.95	498.58[b]
	SD	7.29	2.02	2.30	0.10	47.12

Explanations: a, b – differences statistically significant at $P \leq 0.05$

Tab. 7. Chemical composition and energy value of ham in relation to fattening pig genotype at the *MYOG* locus

MYOG genotype		Water (%)	Fat (%)	Protein (%)	Collagen (%)	Energy value (kJ/100 g)
AA	$\bar{x}$	73.72	3.06	21.37	1.25	476.51
	SD	5.01	1.97	1.51	0.18	58.47
AB	$\bar{x}$	73.14	3.12	22.24	1.37	493.52
	SD	5.45	1.58	1.82	0.12	62.08
BB	$\bar{x}$	74.12	3.19	20.79	1.20	471.46
	SD	5.52	1.34	1.32	0.11	55.31

Chemical properties of loin and ham. Tables 6 and 7 contain the chemical composition and caloric value of loin and ham of fattening pigs with different genotypes at the *MYOG* locus. The polymorphism of this gene was shown to have a significant effect on the proportion of fat and the energy value of the loin. The fat content of the loin of individuals with the BB genotype was significantly higher ($P \leq 0.05$), by 0.76% and by 0.70%, than in individuals with the AB and AA genotypes, respectively. The energy value of the loin of individuals with genotype BB was 23.7 kJ higher than that of individuals with genotype AB and 32.87 kJ higher than that of individuals with genotype AA.

Kim et al. (10), on the basis of haplotype analysis of the myogenin gene (polymorphism in the 5' and 3' region), showed that the TC/BB haplotype was characterised by quality trait values of RFN meat, the most desired by consumers. Also in our study, a favourable correlation between the BB genotype and the quality of loin and ham was demonstrated. This gives rise to the preliminary conclusion that the myogenin gene is an important genetic factor determining the pig's ability to produce lean meat while maintaining its good quality.

Conclusion. Based on the results obtained, it can be concluded that the polymorphism of the *MYOG* gene had an effect on the slaughter value of carcasses and, above all, on meat quality. It was shown that fattening pigs with the AA genotype, compared to AB heterozygous individuals, were characterised by the highest value of loin eye height. Moreover, these homozygotes were characterised by the highest weights of whole ham and ham without skin. With regard to meat quality, a significant effect of the polymorphism at the *MYOG* locus on the value of water holding capacity was shown in both loin and ham. The loins and hams obtained from carcasses of fatteners with the AA genotype contained the highest amount of free water (WHC). Of the chemical components analysed, a significant effect of genotype at the *MYOG* locus on fat content was shown only in the loin. The highest proportion of fat was characteristic of individuals with genotype BB. At the same time, the loin of these fatteners (BB) proved to be the most caloric.

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