

An association between the *MyoD* gene polymorphisms and carcass traits in two- and three-breed crossbred pigs*

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The objective of the study was to analyse the relations between the porcine *MyoD* genotypes and carcass traits in two-breed (Polish Large White × Polish Landrace) and three-breed (Polish Large White × Polish Landrace sows mated to Pietrain boars) crossbreds both being the most common in Poland. A total of 113 animals (78 two- and 35 three-breed crossbreds) were included. The genotypes were defined at the following *MyoD* loci: *MYOD1* (C489T), *MYF5* (A65C) and *MYF6* (C920G and A942C). Dissection of carcass-sides was performed according to the method recommended by Walstra and Merkus in 1995. The CC genotype at the *MYOD1* locus appeared to be most favourable for some traits characterizing carcass muscling, independently on a type of crossbred and similarly to that observed in authors' earlier study on pure PLW and PL breeds. The effect of different genotypes at *MYF5* and *MYF6* loci on a value of certain carcass traits was found to be affected by the type of crossbred. It may suggest that the SNPs in these genes are not causative mutations, but might be considered as genetic markers of other mutations influencing the analysed carcass traits.

KEY WORDS: carcass / gene polymorphism / *MyoD* genes / pig

Meat deposition capacity is related to muscle fibre numbers. Muscle fibre formation takes place during embryonic development regulated by the *MyoD* gene family,

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consisting of *MYOD1*, *MYF5*, *MYOG* and *MYF6* genes [Te Pas and Visscher 1994]. During muscle development, each of the *MyoD* family genes has a distinct temporal pattern of expression [Buckingham 1992]. They control entire muscle development – from commitment and proliferation of muscle precursor cells, through muscle fibre formation and their postnatal maturation and functions [Te Pas and Visscher 1994]. Thus, these genes were selected as candidate genes potentially affecting carcass quality. An association between polymorphism in the non-coding region of *MyoD* genes and carcass traits has been analysed within several breeds and crosses [Te Pas *et al.* 1999, Cieślak *et al.* 2000, 2002, Kurył *et al.* 2002]. A significant difference in a value of several carcass traits was observed between various *MyoD* genotypes. However, one gene variant beneficial for a trait in particular breed appeared to be undesirable for the same trait in another breed. It suggested, that gene mutations being analysed, could not be causal mutations for these differences in carcass traits level. The effect of polymorphisms discovered in the coding or 5'-flanking regions of *MYOD1* and *MYF5* genes [Urbański and Kurył 2004ab] on carcass traits appeared similar irrespective of the animals' breed [Urbański *et al.* 2005] or line [Urbański *et al.* 2006]. However, an absence or a very low frequency of one of homozygous genotypes made it difficult to perform a proper analysis because a comparison of trait value between two homozygous genotypes was impossible.

The purpose of the present study was to analyse an association between *MyoD* genotypes and selected carcass traits in two groups of crossbreds: Polish Large White × Polish Landrace and (Polish Large White × Polish Landrace) × Pietrain, both belonging to the most common pig crossbreds in Poland.

Material and methods

The analysis was conducted on 113 pigs of both sexes reared in Broniewo and Agro-Wronie farms (Kujawy region). Both maintenance and feeding were similar for all animals and remained in accordance with obligatory standards. The pigs were crossbred progeny of Polish Large White (PLW) × Polish Landrace (PL) – 78 animals (7 families) and of PLW × PL sows mated to Pietrain sires – 35 animals (5 families). At a live body weight of about 105 kg the animals were slaughtered and right carcass-sides were dissected according to Walstra-Merkus method [Walstra and Merkus 1995]. In 19 PLW × PL crossbreds some carcass characteristics (e.g. composition of loin) were not evaluated.

Genomic DNA was isolated from leukocytes as recommended by Kawasaki [1990]. The *RYR1/HinP1* genotypes were identified using sequence of primers according to Kamiński *et al.* [2001], whereas those at *MYOD1* and *MYF5* loci – following Urbański and Kurył [2004ab]. The *MYF6* genotypes were defined following Wszyńska-Koko and Kurył [2004] and Wszyńska-Koko *et al.* [2004].

Association analyses were carried out separately for each type of crossbred, using the least squares method of the GLM procedure [SAS 8.2, 2002]. The model included

effects of the *RYR1* genotype, sex and sire. The intra-family comparative analyses were also performed. Age at slaughter and weight of right carcass side were included as covariates. Statistical analyses included:

- mean daily live weight gain;
- weight of ham (WH);
- weight of loin (WL);
- weight of sirloin (WSL);
- weight of meat of ham (WHM);
- weight of meat of loin (WLM);
- weight of meat of valuable cuts (WMVC);
- loin eye area (LA)
- meat content of ham (MCH);
- meat content of loin (MCL);
- meat content of carcass (MCC).

Results and discussion

Our earlier studies as well as those by other authors [Kurył 1998, a review] showed that *RYR1* genotype affects the carcass traits significantly and may modify the effect of other genes. Therefore, in the present study, an effect of *RYR1* genotype was included into the statistical model applied. The frequency of heterozygotes *CT* was found relatively low and only 5.4% and 15% of animals were of this genotype within *PLW* × *PL* and (*PLW* × *PL*) × *Pietrain* crosses, respectively. The homozygotes of *RYR1^T* allele were not present. Carcass traits analysed in this study did not differ between animals of *CT* and *CC* genotype at the *RYR1* locus (data not shown).

The families within both types of crossbreds have been arranged through mating of heterozygous parents concerning individual *MyoD* loci in order to obtain three possible genotypes at this locus within progeny of the same parents. Unfortunately, homozygotes *TT* at locus *MYOD1* and genotypes *GC/GC* at locus *MYF6* were still represented by a low number of animals (Tab. 1 and 2).

A significant relation was observed between several carcass traits and the genotype at the *MYOD1*, *MYF5* and *MYF6* loci. In Tables 1 and 2 only significant relations between *MyoD* genotype and carcass traits are shown. The transition *C489T* in exon 1 of *MYOD1* gene appeared to be significant for loin traits in both types of crossbreds. Homozygotes *CC* showed a significantly greatest loin eye area (LA) within *PLW* × *PL* crossbreds (Tab. 1). The difference between homozygotes amounted to 6 cm². However, a low number of animals of *TT* genotype might affect this result. A similar relation was found between *MYOD1* genotypes regarding *C489T* transition and two loin traits (WLM and MCL) in three-breed crossbreds (Tab. 2). The highest value of these traits was observed in *CC* genotype. Moreover, meat content of carcass evaluated with the Walstra-Merkus method [1995] was also highest in these animals.

Table 1. Least squares means and their standard errors (\pm) for carcass traits in Polish Large White \times Polish Landrace pigs as related to *MyoD* loci

Locus/ mutation	Genotype	n	WH (kg)	WHM (kg)	WLM (kg)	LA (cm ²)	WSL (g)
<i>MYOD1</i>	<i>CC</i>	21				43.7 ^A \pm 1.6	
<i>C489T</i>	<i>CT</i>	30	ns	ns	ns	41.8 ^a \pm 1.5	ns
	<i>TT</i>	8				37.5 ^{Bb} \pm 1.9	
<i>MYF5</i>	<i>AA</i>	20	9.83 ^a \pm 0.16	6.85 ^a \pm 0.18			
<i>A65C</i>	<i>AC</i>	33	9.59 ^b \pm 0.10	6.65 ^{ab} \pm 0.12	ns	ns	ns
	<i>CC</i>	25	9.56 ^b \pm 0.12	6.52 ^b \pm 0.14			
<i>MYF6</i>	<i>CA/CA</i>	30			3.68 ^a \pm 0.11		375.4 ^a \pm 14.3
<i>C920G</i>	<i>CA/GC</i>	26	ns	ns	3.81 ^b \pm 0.11	ns	402.8 ^b \pm 14.1
<i>A942C</i>	<i>GC/GC</i>	3			3.38 ^{ab} \pm 0.28		384.4 ^{ab} \pm 36.8

^{aA...} Within columns means bearing different superscripts differ significantly at: small letters – $P < 0.05$; capitals – $P < 0.01$.

WH – weight of ham; WHM – weight of ham meat; WLM – weight of loin meat; LA – loin eye area; WSL – weight of sirloin.

Table 2. Least squares means and their standard errors (\pm) for carcass traits in (Polish Large White \times Polish Landrace) \times Pietrain pigs as related to *MyoD* loci

Locus/ mutation	Geno- type	n	WMVC (kg)	MCC (%)	WL (kg)	WLM (kg)	MCL (%)	LA (cm ²)
<i>MYOD1</i>	<i>CC</i>	12	25.9 ^a \pm 0.5	60.6 ^a \pm 1.3		4.95 ^a \pm 0.16	59.68 ^a \pm 2.01	
<i>C489T</i>	<i>CT</i>	17	24.2 ^a \pm 0.5	56.5 ^b \pm 1.2	ns	4.48 ^b \pm 0.15	54.04 ^b \pm 1.71	ns
	<i>TT</i>	6	24.9 ^{ab} \pm 0.6	57.8 ^{ab} \pm 1.4		4.68 ^{ab} \pm 0.18	57.03 ^{ab} \pm 1.81	
<i>MYF5</i>	<i>AA</i>	8			8.12 ^a \pm 0.22			
<i>A65C</i>	<i>AC</i>	15	ns	ns	8.66 ^b \pm 0.21	ns	ns	ns
	<i>CC</i>	12			8.28 ^{ab} \pm 0.18			
<i>MYF6</i>	<i>CA/CA</i>	16				4.78 ^a \pm 0.13		53.14 ^a \pm 2.43
<i>C920G</i>	<i>CA/GC</i>	18	ns	ns	ns	4.64 ^b \pm 0.15	ns	49.61 ^b \pm 2.65
<i>A942C</i>	<i>GC/GC</i>	1				4.64 ^{ab} \pm 0.43		50.81 ^{ab} \pm 7.83

^{Aa...} Within columns means bearing different superscripts differ significantly at $P < 0.05$.

WMVC – weight of meat of valuable cuts; MCC – meat content of carcass; WL – weight of loin; WLM – weight of loin meat; MCL – meat content of loin; LA – loin eye area.

Higher values of some traits characterizing carcass muscling were observed earlier in *CC* as compared to *CT* or *TT* animals within pure breeds and synthetic Line 990 [Urbański et al. 2005, 2006].

A comparison of selected carcass traits between animals of different genotypes at nucleotide 65 in the 5' flanking region of the *MYF5* gene showed a highest weight of ham (WH) and weight of ham meat (WHM) for *AA* homozygotes being crosses PLW \times PL (Tab. 1), whereas among three-breed crosses (PLW \times PL) \times P a significantly highest weight of loin (WL) was observed in heterozygotes (Tab. 2). In purebred

PLW and PL pigs, as well as in Line 990, the genotype *AA* appeared to be the most advantageous for WL, whereas the *CC* genotype for loin eye area (LA) – Urbański *et al.* [2005, 2006].

A significant relation was also found between polymorphisms identified in exon 1 of the *MYF6* gene (*C920G* and *A942C*) and values of some carcass traits. Weight of loin meat (WLM) and weight of sirloin (WSL) in heterozygotes were both significantly higher than in homozygotes *CC/AA* (at nucleotides 920 and 942, respectively) being crosses PLW × PL (Tab. 1). A low number of *GG/CC* animals (3 individuals only) excluded the comparison of homozygotes *CC/AA* and *GG/CC* at nucleotides mentioned above. These polymorphisms in the *MYF6* gene also affected two carcass traits in three-breed crossbreds. Weight of loin meat (WLM) and loin eye area (LA) were significantly higher in homozygotes *CC/AA* at the nucleotides 920 and 942 than in *CG/AC* heterozygotes. These results suggest that both SNPs in *MYF6* gene are not causative mutations because of an opposite effect of the same genotype at the *MYF6* locus (*CA/CA*) on a value of particular carcass trait (weight of loin), observed within analysed two- and three-breed crossbreds. These SNPs can be considered only as markers of other mutations that affect a value of considered carcass trait. Moreover, only three and one *GG/CC* genotypes were found in two- and three-breed crossbreds, respectively. Thus, a comparative analysis between all *MYF6* genotypes, similar to that presented by Wyszzyńska-Koko *et al.* [2006] was not possible because of too low representativeness of some genotypes.

Summarizing the analysis performed on two- and three-breed crossbreds one can conclude that *MYOD1* genotype regarding *C489T* polymorphism might be most useful in pigs selection directed towards increasing the meat content of carcass compared to remaining SNPs in *MyoD* genes considered in this study. Its effect on a value of some traits appeared to be universal, independent on breed or type of crossing.

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Zależność między polimorfizmem genów *MyoD* a cechami tuszy dwu- i trójrasowych mieszańców świń

Streszczenie

Analizowano wpływ polimorfizmu genów z rodziny *MyoD* – *MYOD1*, *MYF5* i *MYF6* – na cechy charakteryzujące mięsność tuszy 78 mieszańców dwurasowych wbp × pbz i 35 mieszańców trójrasowych (wbp × pbz) × pietrain. Półtusze dysekowano metodą Walstry i Merkusa (wg. standardów UE). W modelu statystycznym uwzględniono stały efekt genotypu *RYR1* i płci. U obu typów mieszańców potwierdzono obserwowany wcześniej w rasach wbp i pbz korzystny wpływ genotypu *CC* względem *locus MYOD1* (*C489T*) na niektóre cechy mięsności tuszy. Natomiast pozytywny wpływ poszczególnych genotypów *MYF5* (*A65C*) i *MYF6* (*C920G* i *A942C*) był różny w zależności od typu mieszańców. Podobnego typu zależności w odniesieniu do genotypów *MYF5* i *MYF6* odnotowano we wcześniejszych badaniach przeprowadzonych na świnich czystych ras wbp i pbz. Sugeruje to, że genotyp *MYOD1* (*C489T*) można by wykorzystywać w selekcji ukierunkowanej na zwiększenie zawartości mięsa w tuszy, a analizowane SNPs w genach *MYF5* i *MYF6* nie mogą być rozpatrywane jako mutacje przyczynowe, ale jako markery innych mutacji, wpływających na wartość analizowanych cech.

