

Expression profile of *MYF5* and *MYF6* genes in skeletal muscles of young growing gilts of five breeds at different ages, based on the most stable reference genes

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The expression profile was evaluated of *MYF5* and *MYF6* genes in skeletal muscles of young growing Polish Large White (PLW), Polish Landrace (PL), Pietrain (PIE), Duroc (DUR) and Pulawska (PUL) gilts at different ages. Normalization of *MYF5* and *MYF6* was performed on reliable porcine reference genes (PRGs), where expression stabilities of nine of them (*ACTB*, *B2M*, *GAPDH*, *SDHA*, *HPRT1*, *RPL13A*, *YWHAZ*, *TBP*, *TOP2B*) were evaluated by RT-qPCR method and NormFinder

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software. Results revealed *HPRT1*, *TBP* and *TOP2B* as highly stable and PRGs. The age-dependent and breed-specific skeletal muscle expression comparisons revealed highly significant ($P < 0.01$) differences in *MYF6* expression levels of all skeletal muscles among investigated breeds. *MYF6* gene expression in PIE and DUR were higher compared to PLW, PL and PUL gilts. Contrarily, paired-wise comparison of *MYF5* gene expression showed only significant difference between DUR and PUL for *semimembranosus*, and PL and PLW, DUR and PL, PIE and PL, DUR and PUL and PIE and PUL for *gluteus medius* muscle. There was no significant relationship identified between gilt ages and the level of expression of *MYF5* and *MYF6* genes. However, their highest expression was identified in *longissimus dorsi* followed by *gluteus medius* and *semimembranosus* muscles. It is concluded that normalization of gene expression has to be done on more than one PRG to reduce the errors in transcription level estimates. Moreover, significantly different breed-specific expression of porcine *MYF5* and *MYF6* allowed the authors to prioritize these genes as potential candidate genes for trait-associated study.

KEY WORDS: breed / development stage / gen expression / muscle / pig / RT-qPCR

Real-time quantitative polymerase chain reaction (RT-qPCR) enables rapid and reliable quantification of mRNA transcription level [Bustin 2000]. However, it requires several steps of optimization. The first critical point in this method is validation of internal control genes for comparison of target gene expression levels. The validation of the control genes selected as reference is based upon variability in their expression among samples. Such variation of reference genes can introduce or mask expression differences among the genes of interest, rendering study result meaningless [Dheda *et al.* 2005]. The usage of porcine reference genes (PRGs) is necessary for reducing differences arising from various amount of cDNA template in individual samples. Variation of the cDNA template amount would be caused by sample preparation, quality of mRNA obtained from tissue, as well as efficiency of extraction and reverse transcriptions. Proper normalization of target gene expression is essential for the application of RT-qPCR in gene expression studies. Among several strategies proposed, reference genes are commonly accepted and frequently used to normalize RT-qPCR [Ginzinger 2002, Huggett *et al.* 2005]. In the present report the main aim was investigation of *MYF5* and *MYF6* genes expression in three pig muscles on the basis of a set of nine PRGs, previously examined on liver tissue [Pierzchała *et al.* 2011]. The muscle expression of particular genes such as myogenic transcription factors could significantly affect the meat content in carcass, as well as meat quality. Therefore, analysis of genes transcription would provide valuable information about genetic background of leanness and meat quality, what is essential for meat production.

Several authors have found correlations between muscle fibre type or size and meat quality traits in pigs [Chang *et al.* 2003, Ryu and Kim 2005]. The postnatal muscle growth and differentiation is characterized by many cellular and metabolic events related to myogenesis and controlled by different factors [Pierzchała *et al.* 2011, in press]. Myogenesis is mainly controlled by genes of *MyoD* family, which encodes the basic helix-loop-helix (bHLH) proteins that initiate the formation of muscle fibre and regulate the transcription of muscle specific genes [Te Pas *et al.* 2007]. The *MyoD*

gene family comprises of four structurally and functionally related genes: *MYOD1* (*MYF3*), *MYOG* (myogenin), *MYF5* and *MYF6* (herculin) – Olson *et al.* [1991], Weintraub *et al.* [1991]. Each gene of this family is composed of three exons and share homology within the region coding for bHLH domain. Expression of these genes affect myogenesis by inducing the cascade expression of other muscle-specific genes [Lassar *et al.* 1989] and participating in precise regulation of the balance between proliferation and differentiation of primary muscle cells [Kitzmann and Fernandez 2001].]Therefore, genes of *MyoD* family are considered as candidate genes (CGs) for meat production traits [Te Pas *et al.* 1999b, Wyszynska-Koko *et al.* 2006, Verner *et al.* 2007].

The porcine *MYF5* and *MYF6* genes exhibit a number of polymorphisms [Ernst *et al.* 1994, Cieslak *et al.* 2002, Vykoukalova *et al.* 2003, Urbanski and Kuryl 2004, Wyszynska-Koko and Kuryl 2004, Wyszynska-Koko *et al.* 2006]. However, their associations with growth, or carcass and meat quality traits were found mostly negative or not consistent [Stratil and Cepica 1999, Te Pas *et al.* 1999a, Urbanski *et al.* 2006]. Therefore the aim of this study was to explain role of expression profile of *MYF5* and *MYF6* genes in skeletal muscles of growing pigs. The study material composed of young gilts of five breeds and at different ages.

Material and methods

Animals and sampling

The investigation design involved 180 gilts of five breeds, Polish Large White (PLW), Polish Landrace (PL), Petrain (PIE), Duroc (DUR) and Pulawska (PUL) slaughtered at the age of 60, 90, 120, 150, 180 and 210 days. Each of the breeds were represented by 36 gilts, 6 gilts of each breed were slaughtered at each particular age. Samples were taken from *m. longissimus dorsi* (LD), *m. gluteus medius* (GM) and *m. semimembranosus* (SM) directly post-slaughter, immediately snap-frozen in liquid nitrogen and stored at -80°C until RNA isolation. Treatment and slaughtering of animals were in accordance with the guiding principles for the care and use of research animals.

RNA extraction and reverse transcription

Total RNA was isolated from muscle samples using Trizol reagent (INVITROGEN, USA) according to Chomczynski and Sacchi [1987]. The contamination of genomic DNA was removed by treating total RNA with RNase-free DNase (PROMEGA, USA), according to the manufacturer's recommendations. The quantity and quality of total RNA preparations were evaluated spectrophotometrically at 260 nm (NANODROP, USA). The purity of total RNA was determined by the A260/280 and A260/230 ratio and its integrity was checked electrophoretically using 1% formaldehyde denaturing gel. The single strand (ss) cDNA was synthesized using M-MLV reverse transcriptase (PROMEGA, USA) in a 25 µl reaction mixture according to the manufacturer's

instructions. The reverse transcription PCR reaction (RT-PCR) was performed for 1 h at 42°C in 60 µl of mixture containing 2 µg of total RNA, 50 mM Tris–HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, 0.3 mM dNTP mix, 25U of RNase inhibitor, 0.5 µg of oligo (dT)15 primer, and 200U of M-MLV reverse transcriptase (PROMEGA, USA).

Real-time PCR quantification with SYBR green

Nine PRGs belonging to various functional classes were selected. This set of genes commonly used as references for reaction of RT-qPCR [Pierzchala *et al.* 2011]. The primers for target genes (*MYF5* and *MYF6*) for real time PCR analysis were designed (Tab. 1) for the sequences of second and third exon of the *MYF5* and *MYF6* genes using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The PCR amplification was performed in a LightCycler ® 480 real time PCR system (ROCHE APPLIED SCIENCE) using 96-well optical plates with a SYBR green I master mix (ROCHE APPLIED SCIENCE). A PCR reaction contained forward and reverse primers (10 µM), cDNA (100 ng) and SYBR green I master mix. The amplification programme was: 5 min of denaturation at 95°C, 40 cycles of four segment amplification with 10 s at 95°C (denaturation), 10 s at 58–60°C (annealing), and 10 s at 72°C (elongation). Annealing temperatures were optimized for individual genes and primers. The last step of RT-PCR was melting, added to ensure that specific PCR product was obtained. This melting step consisted of 5 s at 95°C, 5 s at 64°C, and slow heating at a rate of 0.1°C per s up to 95°C, with continuous (5 times per 1°C) fluorescence measurement, finally followed by cooling down to 4°C. The efficiency of amplifications of RT-qPCR reactions was estimated basing on serial cDNA template dilutions: 1/4, 1/16, 1/64 and 1/256.

Table 1. Primer sequences, amplicons length, annealing temperature and reference GeneBank accession numbers of porcine target genes

| Gene symbol | Gene name | Primer sequence (5' → 3') | Amplicon length (bp) | Annealing temp. (°C) | GeneBank accession number |
|-------------|---------------------------------------|--|----------------------|----------------------|---------------------------|
| MYF-5 | Myogenic regulatory factor | GAAAGAACAGCAGTTTGTGACA CTCCTGGCAGAAATGGTC | 273 | 59 | [GenBank: Y17154] |
| MYF-6 | Myogenic regulatory factor (herculin) | CTTGAGGGTGCGGATTTC CTCGAGGCTGACGAATCAA | 122 | 60 | [GenBank: AY327443] |

Calculation of Ct value

Variation of genes expression was calculated based on a cycle threshold (Ct) using the LightCycler 480 software (ROCHE DIAGNOSTICS) following the manufacturer's instructions. The Ct value of and mean efficiency of each amplicon were used to calculate their relative expression levels.

Analysis of reference genes expression stability

For stability comparison of candidate PRGs, Visual Basic Application (VBA) tool for Microsoft Excel: NormFinder (<http://www.multid.se/genex/hs410.htm>) were used [Andersen *et al.* 2004]. The NormFinder software utilizes a model-based approach to identify the optimum normalization of genes among a set of candidates. This approach based on mathematical model of gene expression permitted to estimate the intra- and inter-group variations which could be combined into a stability value. The candidate PRGs with minimum intra-group variation will show the highest stability and, therefore, top ranked values [Andersen *et al.* 2004].

Statistical

Three PRGs – *TBP*, *TOP2B* and *HPRT1*– were used to normalize *MYF5* and *MYF6* mRNA levels for porcine skeletal muscles. The statistical analysis aiming at comparing the breed- and age-specific gene expressions of *MYF5* and *MYF6* target genes in skeletal muscles of growing gilts was conducted according to the SAS generalized linear model procedure (PROC GLM). To compare the relative expression levels of porcine *MYF5* and *MYF6* genes the following linear model were used (SAS/STAT 9.22, SAS Institute Inc., Cary, NC, USA):

$$Y_{ijkl} = \mu + d_i + b_j + a_k + m_l + ba_{jk} + \beta w_{ijkl} + e_{ijkl}$$

where:

- d_i – fixed effect of i -th dam;
- b_j – fixed effect of j -th breed;
- a_k – fixed effect of k -th age;
- m_l – effect of l -th muscle;
- ba_{jk} – effect of j -th breed x k -th age interaction;
- β – regression coefficient of covariate traits: weight of right carcass-side;
- e_{ijkl} – random error.

Results and discussion

NormFinder reference genes expression stability analysis

NormFinder [Andersen *et al.* 2004] identified the following most stable PRGs: *B2M*, *HPRT1*, *TOP2B*, *GAPDH*, *ACTB* and *TBP* for LD muscle, *B2M*, *HPRT1*, *ACTB*, *TOP2B*, *GAPDH* and *TBP* for SM, and *TOP2B*, *HPRT1*, *GAPDH*, *ACTB*, *B2M* and *TBP* for GM muscle (Fig. 1, Tab. 2).

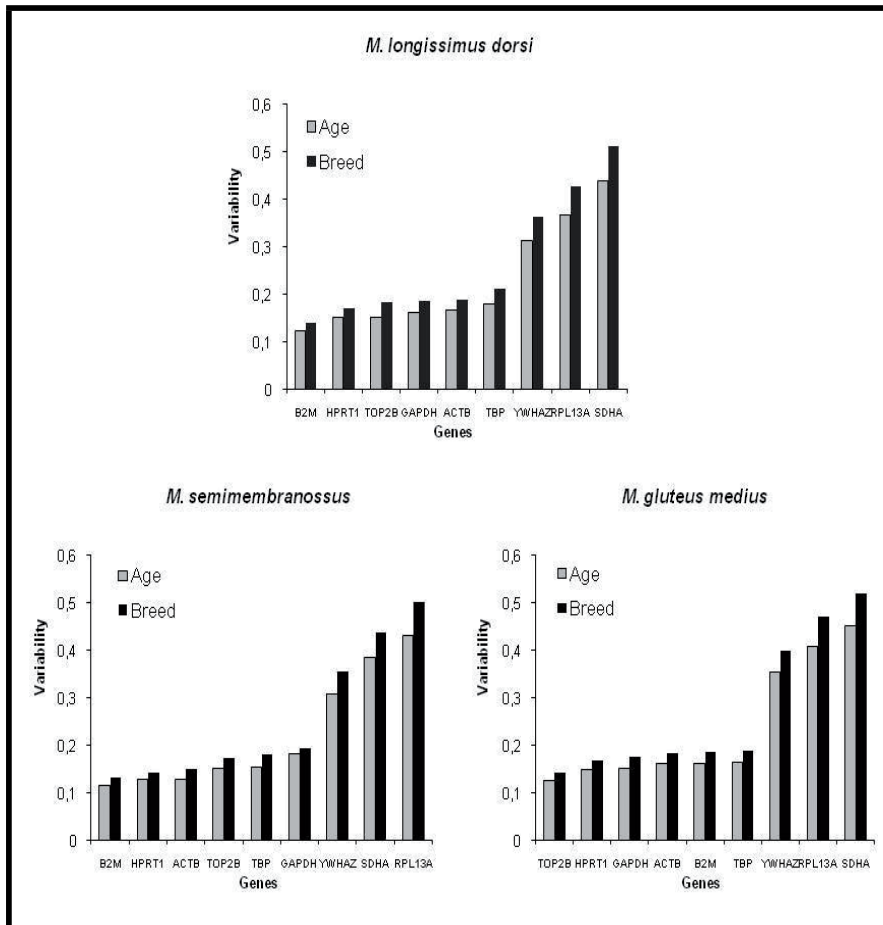


Fig. 1. The mRNA expression stability indices of PRGs according to NormFinder values for porcine skeletal muscles with reference to age and breed.

Breed- and age-specific gene expression of MYF5 and MYF6 target genes (TG) in muscles

The expression profiles of porcine *MYF5* and *MYF6* target genes were obtained by normalizing their expression with *TBP*, *TOP2B* and *HPRT1* PRGs, ranked as high stable in *NormFinder* analysis. The expression of *MYF5* and *MYF6* showed a significant difference in expression level in all investigated porcine skeletal muscles. Moreover, the *MYF6* expression in LD showed levels significantly different from those found in SM (Tab. 3). The relative mRNA transcription level showed higher expression of *MYF6* than of *MYF5* in all muscles (Fig. 2). The high differences between the expression of *MYF5* and *MYF6* were due to the fact, that the former were primarily

Table 2. Stability of the PRGs estimated according to intra- and inter-group variation based on NormFinder approach

| NormFinder age group | | | | | | |
|----------------------|---------|-----------------------------|---------|----------------------------|---------|--------------------------|
| gene symbol | ranking | <i>m. longissimus dorsi</i> | ranking | <i>m. semi-membranosus</i> | ranking | <i>m. gluteus medius</i> |
| <i>TBP</i> | 5 | 0.18 | 4 | 0.16 | 3 | 0.16 |
| <i>TOP2B</i> | 2 | 0.15 | 3 | 0.15 | 1 | 0.13 |
| <i>HPRT1</i> | 2 | 0.15 | 2 | 0.13 | 2 | 0.15 |
| <i>ACTB</i> | 4 | 0.17 | 2 | 0.13 | 3 | 0.16 |
| <i>GAPDH</i> | 3 | 0.16 | 5 | 0.18 | 2 | 0.15 |
| <i>SDHA</i> | 8 | 0.44 | 8 | 0.39 | 6 | 0.45 |
| <i>RPL13A</i> | 7 | 0.37 | 7 | 0.43 | 5 | 0.41 |
| <i>YWHAZ</i> | 6 | 0.31 | 6 | 0.31 | 4 | 0.35 |
| <i>B2M</i> | 1 | 0.12 | 1 | 0.12 | 3 | 0.16 |
| NormFinder age group | | | | | | |
| gene symbol | ranking | <i>m. longissimus dorsi</i> | ranking | <i>m. semi-membranosus</i> | ranking | <i>m. gluteus medius</i> |
| <i>TBP</i> | 4 | 0.19 | 2 | 0.14 | 3 | 0.18 |
| <i>TOP2B</i> | 2 | 0.17 | 3 | 0.15 | 2 | 0.17 |
| <i>HPRT1</i> | 1 | 0.14 | 1 | 0.13 | 4 | 0.19 |
| <i>ACTB</i> | 3 | 0.18 | 6 | 0.19 | 2 | 0.17 |
| <i>GAPDH</i> | 7 | 0.43 | 9 | 0.50 | 6 | 0.47 |
| <i>SDHA</i> | 8 | 0.51 | 8 | 0.44 | 7 | 0.52 |
| <i>RPL13A</i> | 5 | 0.21 | 5 | 0.18 | 4 | 0.19 |
| <i>YWHAZ</i> | 4 | 0.19 | 4 | 0.17 | 1 | 0.14 |
| <i>B2M</i> | 6 | 0.36 | 7 | 0.36 | 5 | 0.40 |

Table 3. Fold of change and relative mRNA expression levels (least squares means and standard errors) of MYF5 and MYF6 gene expression in relation to skeletal muscles of pigs

| Skeletal muscle / Target gene | MYF5 | MYF6 | MYF5/MYF6 |
|-------------------------------|------------------------------|-----------------------------|-----------------------------|
| <i>M. longissimus dorsi</i> | 0.0584 ^A ±0.0080 | 2.8950 ^A ±0.4818 | 83.98 ^a ±50.99 |
| <i>M. semimembranosus</i> | 0.0062 ^{AB} ±0.0021 | 1.2905 ^A ±0.1275 | 208.87 ^{aA} ±13.50 |
| <i>M. gluteus medius</i> | 0.0364 ^B ±0.0065 | 1.7133±0.3995 | 56.92 ^A ±41.23 |

^{aA...} Means in columns bearing the same superscripts are significantly different: small letters – P<0.05; capitals – P<0.01.

expressed in the satellite cells. This low expression of MYF5 may be related to the low number of activated satellite cells in porcine skeletal muscles.

Comparison of age groups revealed no significant differences among them, while among breeds, both target genes showed significant differences in their expression level. DUR and PIE showed a higher mean expression level of MYF6 gene in LD and SM in comparison to PLW, PL, and PUL gilts. Moreover, relatively low expression levels of MYF6 in GM were observed in PUL gilts compared to other breeds. In

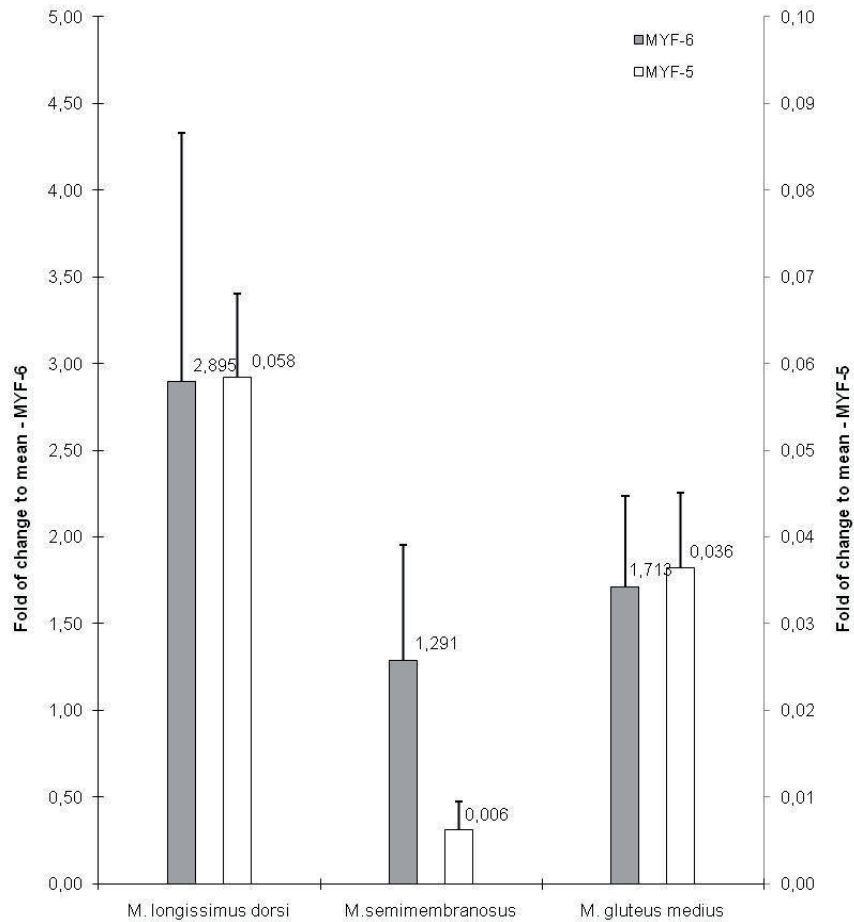


Fig. 2. Comparison of relative mRNA transcription levels between *MYF5* and *MYF6* target genes in porcine skeletal muscles (means and standard deviations).

Table 4. Fold of change in relative mRNA levels (least squares means and standard errors) of *MYF5* and *MYF6* genes in relation to pig breeds

| Breed (total n=180) | <i>M. longissimus dorsi</i> | | <i>M. semimembranosus</i> | | <i>M. gluteus medius</i> | |
|------------------------|-----------------------------|--------------|---------------------------|--------------|--------------------------|--------------|
| | <i>MYF-5</i> | <i>MYF-6</i> | <i>MYF-5</i> | <i>MYF-6</i> | <i>MYF-5</i> | <i>MYF-6</i> |
| Polish Large White | 1.46±0.87 | 1.01±0.55 | 0.51±0.27 | 0.56±0.30 | 1.16±0.67 | 0.88±0.36 |
| Polish Landrace | 1.55±0.81 | 0.93±0.56 | 0.49±0.29 | 0.52±0.28 | 0.93±0.51 | 0.90±0.41 |
| Pulawska | 1.33±0.69 | 0.66±0.43 | 0.39±0.23 | 0.25±0.12 | 0.94±0.42 | 0.57±0.27 |
| Duroc | 1.34±0.73 | 1.99±1.08 | 0.52±0.42 | 0.93±0.41 | 1.19±0.58 | 1.14±0.61 |
| Pietrain | 1.48±0.60 | 2.42±1.49 | 0.43±0.22 | 1.09±0.64 | 1.26±0.61 | 1.14±0.55 |

general, both target genes showed highest expression levels in LD, followed by GM and SM in pigs of all investigated breeds (Tab. 4).

Furthermore, the breed-specific paired-wise comparisons of LSM differences and P-values of target genes expressed in porcine skeletal muscles showed significant ($P<0.05$) and highly significant ($P<0.01$) differences in *MYF6* expression levels of skeletal muscles among investigated breeds (Tab. 5). Highly significant differences in *MYF6* expression level were identified between all investigated breeds, except PL and PLW in all muscles, PUL and PL for LD, and PIE and DUR for GM. Contrarily to *MYF6*, comparison of means mRNA of *MYF5* levels in LD showed no significant effects among investigated breeds. However, significant difference was observed for *MYF5* gene expression in SM muscle between DUR and PUL gilts. For the GM muscle, comparison of breeds showed significant differences between PL and PLW, DUR and PL, PIE and PL, DUR and PUL and PIE and PUL.

The within-breed and age-dependent expression profiles analysis revealed the highest expression of both *MYF5* and *MYF6* target genes in LD, followed by GM and SM muscles. No significant relationship was found between ages (Fig. 3 and 4).

The results obtained may vary due to the different stability even of the same reference genes. Therefore, the use of the reference genes for normalization is a perpetual matter of debate. Because numerous studies have shown that reference genes used are influenced by specific experimental conditions [Warrington *et al.* 2000, Stürzenbaum and Kille 2001]. In this study, we have analysed the reference gene stability and expression levels of *MYF5* and *MYF6* target genes in five pig breeds at different ages of development. This was essential stage for normalization of the target genes relative

Table 5. Among breeds, paired-wise comparisons of *MYF5* and *MYF6* target genes expression in skeletal muscles of pigs. Least squares means differences and *P*-values (italics)

| Gene | Breed | <i>M. longissimus dorsi</i> | | | | | <i>M. semimembranosus</i> | | | | | <i>M. gluteus medius</i> | | | | |
|-------|--------------------|-----------------------------|--------|-------|-------|------|---------------------------|--------|-------|--------|------|--------------------------|--------|-------|-------|-------|
| | | PLW | PL | PUL | DUR | PIE | PLW | PL | PUL | DUR | PIE | PLW | PL | PUL | DUR | PIE |
| MYF-5 | Polish Large White | | 0.61 | 0.44 | 0.48 | 0.92 | | 0.68 | 0.05 | 0.87 | 0.21 | | 0.05 | 0.08 | 0.82 | 0.41 |
| | Polish landrace | 0.088 | | 0.21 | 0.22 | 0.73 | -0.025 | | 0.12 | 0.56 | 0.43 | -0.229 | | 0.93 | 0.04 | 0.01 |
| | Pulawska | -0.133 | -0.221 | | 0.94 | 0.41 | -0.120 | -0.095 | | 0.03 | 0.55 | -0.219 | 0.010 | | 0.05 | 0.01 |
| | Duroc | -0.122 | -0.210 | 0.011 | | 0.42 | 0.010 | 0.035 | 0.130 | | | 0.14 | 0.027 | 0.256 | | 0.62 |
| | Pietrain | 0.014 | -0.074 | 0.147 | 0.136 | | -0.081 | -0.056 | 0.039 | -0.091 | | | 0.100 | 0.329 | 0.319 | 0.073 |
| MYF-6 | Polish Large White | 0.67 | 0.04 | <0.01 | <0.01 | | 0.59 | 0.01 | <0.01 | <0.01 | | 0.81 | 0.01 | 0.01 | 0.01 | |
| | Polish landrace | -0.074 | 0.11 | <0.01 | <0.01 | | -0.044 | 0.01 | <0.01 | <0.01 | | 0.024 | 0.01 | 0.02 | 0.02 | |
| | Pulawska | -0.348 | -0.275 | <0.01 | <0.01 | | -0.308 | -0.264 | <0.01 | <0.01 | | -0.312 | -0.336 | <0.01 | <0.01 | |
| | Duroc | 0.986 | 1.059 | 1.334 | | 0.01 | 0.368 | 0.412 | 0.676 | 0.12 | | 0.261 | 0.237 | 0.573 | | 0.95 |
| | Pietrain | 1.414 | 1.488 | 1.762 | 0.428 | | 0.530 | 0.574 | 0.837 | 0.162 | | 0.262 | 0.237 | 0.573 | 0.001 | |

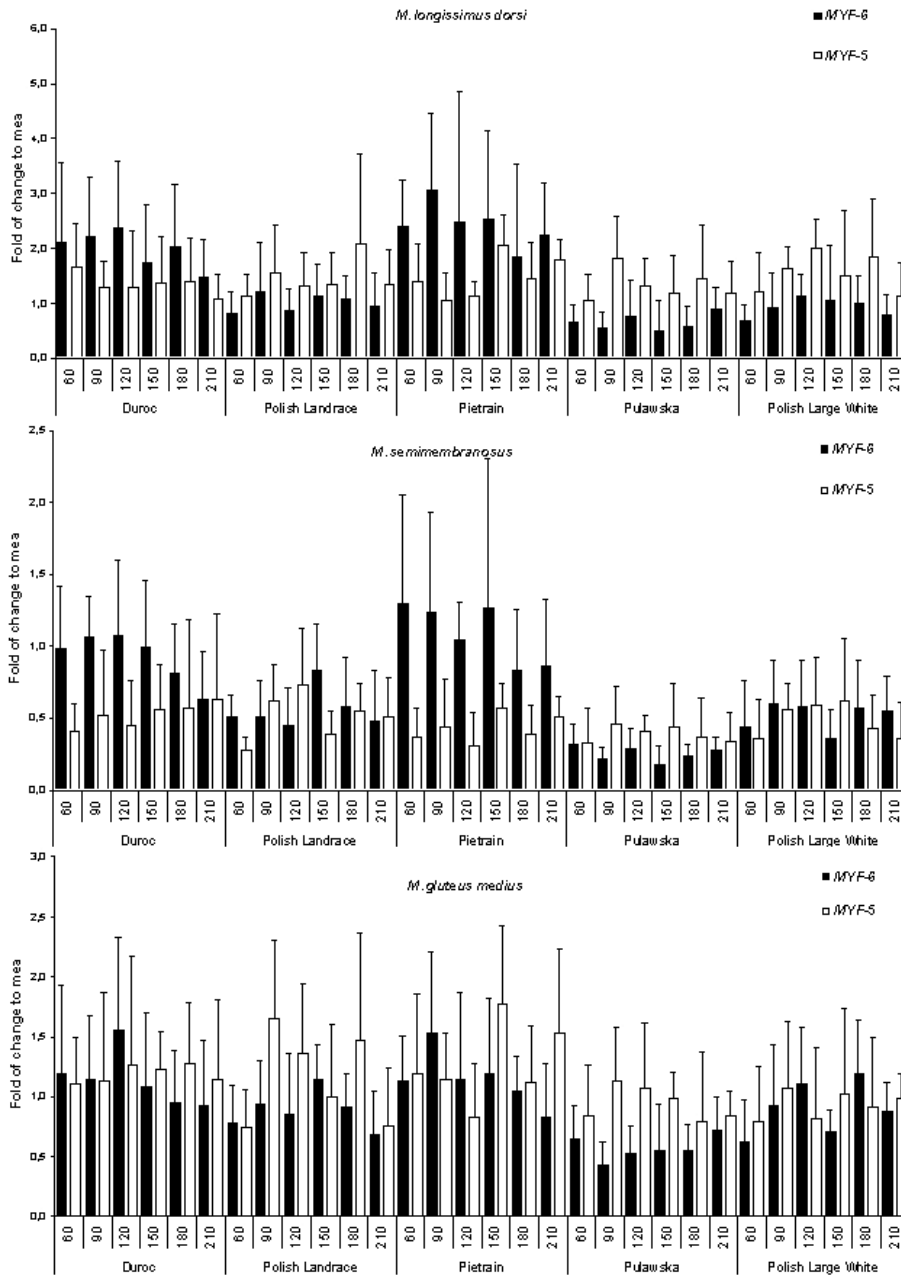


Fig. 3. The mRNA expression levels of the *MYF5* and *MYF6* target genes in porcine skeletal muscles (means and standard deviations).

Expression profile of MYF5 and MYF6 genes

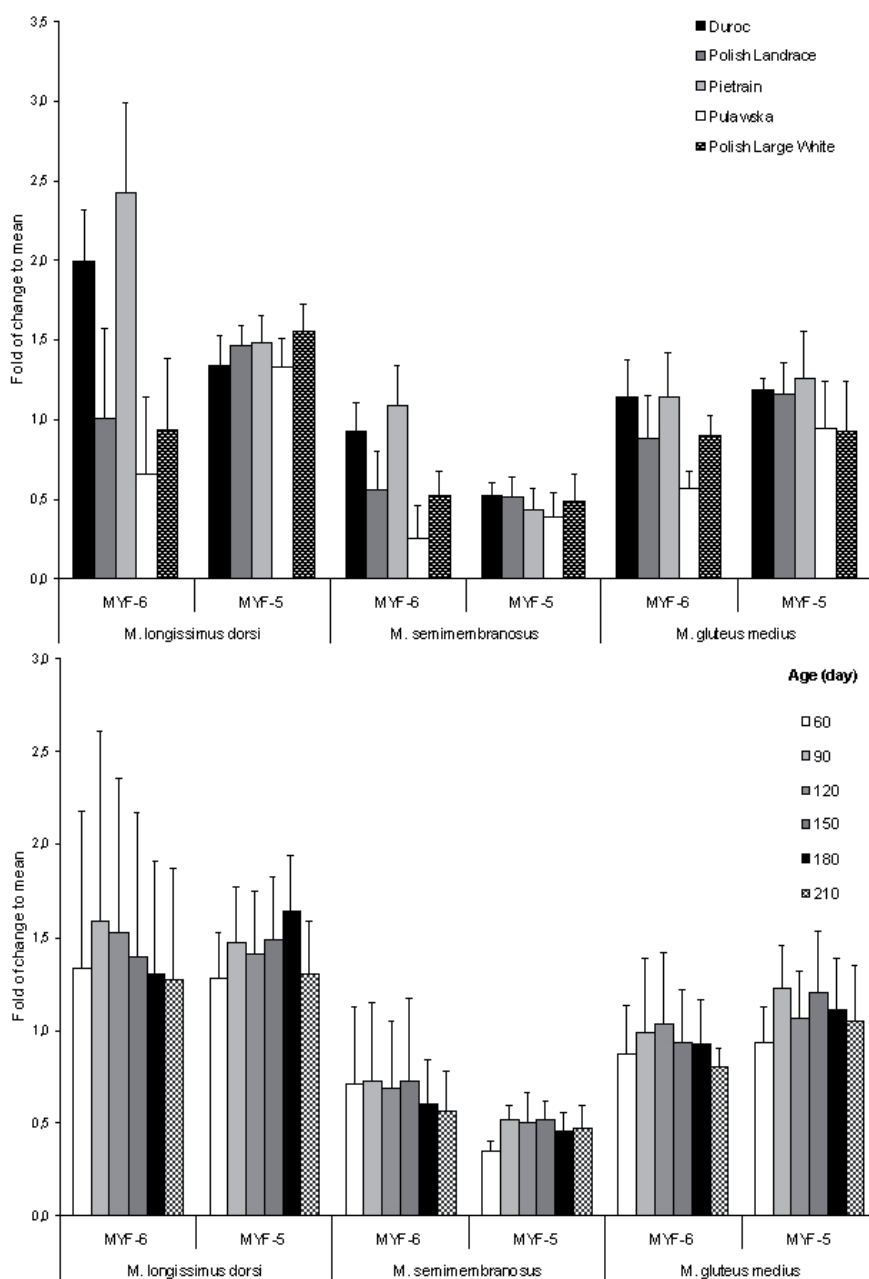


Fig. 4. Breed-specific and age-dependent mRNA expression levels of the *MYF5* and *MYF6* target genes, in porcine skeletal muscles (means and standard deviations).

mRNA levels in RT-qPCR. Therefore, several reference genes were tested according to our specific conditions of investigation [Thellin *et al.* 1999, Pfaffl *et al.* 2004]. In past five years, several reference genes studies dealing with analysis of specific tissues have been carried out on pigs [Erkens *et al.* 2006, Nygard *et al.* 2007, Svobodová *et al.* 2008]. However, most of them were based upon only one reference gene (*GAPDH* or *ACTB*). For more reliable normalization, it is suggested to replace such normalization based on one reference by the multiple reference genes [Vandesompele *et al.* 2002]. The cited authors concluded that the highly stable PRGs like *TBP*, *TOP2B* or *HPRT1* can be recommended simultaneously for normalization for porcine skeletal muscles gene expression studies.

The genomic background underlying breed-specific differences in phenotype of muscle traits is poorly known. The PLW and PL have a little lower muscle mass content in carcass than PIE pigs and are characterized by very good rate of growth and good slaughter parameters. DUR, PIE and PUL pigs are clearly divergent in comparison to PLW and PL. PIE pigs are slower growing breed, but also leaner, whereas DUR and PUL showing relatively high level intramuscular fat content. Moreover, PUL pigs, polish native eco-type breed is characterized by greater backfat thickness, but simultaneously higher quality of meat [Szyndler-Nędza *et al.* 2007]. The carcasses of pigs of different breeds are also divergent in their muscle phenotype traits such as myofibre numbers, size and type. PIE pigs is characterized by hypertrophy of muscle fibers leading to mostly white muscle fiber typing, contrary to this, DUR pigs have smaller, redder fiber types [Sellier, 1998]. The origin of such differences is related to process involving proliferation and differentiation of muscle precursor cells.

In this study, overall postnatal expression of *MYF5* in porcine skeletal muscle showed no significant differences between pig breeds and ages. By contrast, *MYF6* expressed significant differences in transcriptional level of investigated muscles. Recently, significant differences among *MYF6* expression levels in porcine skeletal muscles (*semimembranosus*, *biceps femoris* and *gracilis*) were identified by Ropka-Molik *et al.* [2010]. Among the three ham muscles, they found highest mRNA level of *MYF6* gene in *gracilis* muscle in all breeds, but significant ($P < 0.01$) difference was only found in Pietrains. Our findings are in accordance with those mentioned above, as we found the expression of *MYF6* gene in skeletal muscle of PIE was higher than in gilts of other breeds, and did not demonstrate significant relationship between age and the level of expression of *MYF5* and *MYF6* genes in porcine skeletal muscles [Ropka-Molik *et al.* 2010]. Our results may also suggest that higher *MYF6* gene expression would be related to higher muscularity of carcasses, as it is in PIE breed.

The age-dependent *MYF5* and *MYF6* expression analysis in porcine skeletal muscles showed the highest mRNA level in gilts at the age of 90-150 days of postnatal development in all investigated breeds except *MYF6* that was expressed at highest level in SM muscle at day 60 in PIE and PUL gilts. In general, the age-dependent

changes in populations of proliferating and differentiating satellite cells in porcine skeletal muscle take place between week 1 and 21 of life and at the age from 1 to 7 weeks there is a decrease in percentage of total satellite cells in pigs, which became constant in older animals [Mesires and Doumit 2001].

The age-dependent differences in transcript level might also be breed-specific and caused by different genetic background resulting from different genetic origin. This discrepancy could be explained by breed-specific polymorphisms, such as mutations in *MyoG* gene [Zhu *et al.* 2010]. It is also feasible that another regulation factors would affect expression level of *MYF5* and *MYF6* in gilts of investigated breeds.

The expression analysis of *MYF6* showed that its polymorphism may be important for the myotube fusion, maturation and maintenance of the skeletal muscle weight [Wyszynska-Koko *et al.* 2006]. They identified significant correlation between the *MYF6* polymorphism identified in the promoter region, exon 1 and carcass weight. However, several studies on *MYF5*, *MYF6* showed that their polymorphism does not affect the expression of *MRF* genes [Ernst *et al.* 1994, Stratil and Cepica 1999, Te Pas *et al.* 1999a, Cieslak *et al.* 2002, Vykoukalova *et al.* 2003, Urbanski and Kuryl 2004, Wyszynska-Koko and Kuryl 2004, Urbanski *et al.* 2006]. However, these results suggest that the effects of age and breed-specific expression of *MYF5* and *MYF6* observed in this study could also represent indirect effects of another miogenic regulatory mechanism expressed in porcine skeletal muscle. Nevertheless, the existence of numerous regulatory elements at large distances to *MYF5* and *MYF6* pointed to a very complex pattern of these genes regulation, which show also significant differences between species [Maak *et al.* 2006]. Furthermore, several investigations suggest that *MYF5* and *MYF6* genes may be expressed at a very low level in myofibres (Te Pas *et al.* 2005a, Te Pas *et al.* 2005b, Wyszynska-Koko *et al.* 2006]. Therefore, the observed variation of age/breed-dependent expression of *MYF5* and *MYF6* genes in porcine skeletal muscles may represent a mix of transcriptional activity of satellite cells and myofibres.

This study revealed a newly developed set of PRGs for normalization of mRNA expression data from porcine skeletal muscles. The highly stable PRGs expressed in different tissues provides a basis for possible investigation of candidate genes for postnatal muscle growth, towards improving meat quality. The significantly different expression was presented of *MYF5* and *MYF6* in porcine skeletal muscles. To our knowledge, this study is one of the first analyses of age- and breed-dependent relations of porcine *MYF5* and *MYF6* expressed in postnatal period of skeletal muscle growth. Significant differences between *MYF5* and *MYF6* expressions allowed us to select both candidate genes for further trait-associated studies. The further identification of causal polymorphism and determination of functional role are even more challenging, since there are many different molecular mechanisms through which expression activity of specific genes in myogenic cells can be regulated.

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