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EXPRESSION OF *IGF1* AND *IGF2* GENES IN MUSCLES DURING DEVELOPMENT OF PIGS REPRESENTING FIVE DIFFERENT BREEDS*

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Abstract

Insulin-like growth factors (*IGF1*, *IGF2*) are part of the somatotropic axis – a group of substances involved in controlling many important functions, including muscle growth of mammalian organism. In the study, we analysed changes in the expression level of *IGFs* in muscles during development of pigs (between 60 and 210 days of age). We also compared expression level of *IGFs* between groups of pigs representing five breeds differing in productive traits. Expression level of *IGF2* gene decreased significantly (max. 9-fold) with aging of the animals. Level of *IGF1* also declined but only slightly. Between breed analysis confirmed the effect of *IGF2* G3072A SNP on expression level and indicated the presence of an additional factor influencing expression of *IGF2* gene. Moreover, we observed overexpression of *IGF1* gene in the Pietrain breed at some developmental stages.

Key words: pigs, development, gene expression, *IGF1*, *IGF2*

IGF1 and *IGF2* are important regulators of muscle growth and development (Florini et al., 1996). It was shown that *IGFs* can stimulate proliferation and differentiation of muscle cells (Florini et al., 1986). Patterns of expression of *IGFs*

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during muscle development of farm animals have been studied *in vitro* (reviewed in Oksbjerg et al., 2004; Xi et al., 2007; Kalbe et al., 2008) and to a lesser extent *in vivo* (Gerrard et al., 1998; Stinckens et al., 2007).

Because of their function in muscle development, genes for *IGFs* became candidate genes for meatiness of farm animals. It was proved that the number of CA repeats at intron 1 of *IGF1* gene is positively correlated with plasma *IGF1* concentration and growth and fatness of pigs (Estany et al., 2007). On the other hand, in a non-coding fragment of porcine *IGF2* gene a causative mutation (*IGF2* SNP G3072A), affecting gene expression, weight of muscle and fat deposition, has been identified (Van Laere et al., 2003).

IGF1 is expressed biallelically, whereas *IGF2* is maternally imprinted – in the offspring only paternal allele is expressed (DeChiara et al., 1991). Both of these genes, *IGF1* and *IGF2*, are alternatively spliced in mammals. Recently, two different *IGF1* transcripts (class 1 and class 2) have been cloned and characterized in Songliao black pig (Xiao et al., 2009). Alternative splicing of *IGF2* has been described in humans (Monk et al., 2006), mice (Moore et al., 1997) and cattle (Curchoe et al., 2005; Goodall and Schmutz, 2007).

The aim of our study was to investigate the expression pattern of *IGF1* and *IGF2* genes during postnatal development of muscles in pigs and to determine if there are differences in the expression level of *IGF2* gene between pigs displaying different carcass performance.

Material and methods

The gene expression profile of *IGF1* and *IGF2* was analysed in two muscles: *m. longissimus dorsi* and *m. semimembranosus* of 177 sows from 5 breeds: Duroc, Pietrain, Puławska, Polish Large White and Polish Landrace. Animals were kept in a Pilot Plant of the National Research Institute of Animal Production in Pawłowice under the same housing and feeding conditions. According to the day of slaughter, animals of each breed were divided into 6 age groups (5–6 sows per group): 60, 90, 120, 150, 180 and 210 days old. Animals were related as all sows within a breed had the same father (except the Puławska breed – 3 fathers), and their mothers were sisters. Tissue fragments were collected immediately after slaughter and kept in liquid nitrogen during transportation. Animals of all breeds were stress resistant (*RYR1* C/C) except Pietrain pigs, where C/T heterozygotes occurred.

The total RNA was extracted using TRI-Reagent (Sigma) and Silent Crusher S homogenizer (Heidolph), according to the method described by Chomczyński (1993). The quantity of extracted RNA was estimated by BioPhotometer (Eppendorf), and its quality was evaluated by gel electrophoresis.

The RNA (1 µg) was reverse transcribed into cDNA at 37ºC using High Capacity cDNA Reverse Transcription Kit with random primers (Applied Biosystems), according to manufacturer's protocol.

Primers and probes for *IGF1*, *IGF2*, *18sRNA* and *GAPDH* genes were described previously (Johnson et al., 2002; Van Laere et al., 2003; Applied Biosystems).

Relative Quantification of the expression was performed on 7500 Real-Time PCR System using labelled TaqMan® Tamra probes and TaqMan® Universal PCR Master Mix with UNG AmpErase (Applied Biosystems). Reactions, in a total volume of 50 µl, were performed in duplicate and according to the TaqMan® Universal PCR Master Mix protocol. The protocol included two initial steps: 50ºC for 2 min (UNG incubation) and 95ºC for 10 min (AmpliTaq Gold activation) and 40 cycles of 95ºC for 15 s (denaturation) and 1 min at 60ºC (annealing/extending). *GAPDH* and *18sRNA* were used as an endogenous control. The results were analysed using Sequence Detection System software v. 2.0 (Applied Biosysytems). For statistical analysis, the results obtained for both muscles were combined. Statistical analysis was performed using General Linear Models (SAS Institute) with age and breed as a fixed factor. Genotyping of *IGF2* G3072A SNP was performed on 7500 Real-Time PCR System (allelic discrimination) according to Carrodeguas (2005).

Results

All Duroc, Large White and Landrace animals carried paternally derived A allele in *IGF2*, as the fathers of these groups were A/A homozygotes. There were a few heterozygous pigs which inherited the G allele from the father in the Puławska group, whereas in Pietrain five animals were G/G homozygotes and 10 heterozygotes carried paternally derived G allele. We were unable to assess the origin of the G allele in several animals as both of their parents were heterozygous (Table 1).

Expression of *IGF2* gene declined with age in all breeds except Pietrain. This decrease was the highest in the Duroc breed (9-fold between 60 and 210 days) (Fig. 1). Expression of *IGF1* gene also decreased with age in Large White, Puławska and Duroc but the differences between the youngest and the oldest animals were much lower and statistically significant only in Puławska (Fig. 2).

The Pietrain breed displayed the lowest level of *IGF2* expression at all developmental stages. In young animals the differences were about 2–3 fold whereas at later developmental stages the differences diminished. The highest expression was noted in the Duroc breed at all developmental stages excluding 90 days (Fig. 3). On the other hand, abundance of *IGF1* transcripts was the highest in Pietrain at 90, 120, 150 and 180 days of age. At the age of 210 days Puławska displayed the lowest *IGF1* expression (Fig. 4).

Table 1. Relative Quantity (RQ) values of *IGF2* transcript in muscle. Colour of background indicates *IGF2* G3072A genotype

Breed/Age	60 days	90 days	120 days	150 days	180 days	210 days
Large White	25.84	7.16	13.52	11.49	9.14	3.10
	10.33	28.91	18.77	9.29	5.74	5.51
	25.51	29.56	14.75	11.08	7.65	10.48
	21.92	25.80	14.13	13.90	9.23	4.47
	29.53	18.76	17.43	12.57	8.77	6.21
	8.06	25.18	14.30	11.57	11.93	6.40
Landrace	16.92	15.71	13.71	6.91	2.65	7.66
	18.77	20.38	11.21	4.08	7.51	4.97
	44.55	38.18	19.88	17.66	8.37	8.39
	13.51	13.51	8.67	5.83	3.30	2.26
	28.01	21.31	4.61	12.16	9.06	4.37
	50.45	25.53	21.10	17.28	7.19	8.35
Puławska	16.95	3.55	7.16	5.26	1.13	1.38
	28.13	32.71	18.47	10.91	8.33	4.87
	21.40	23.58	16.48	10.81	8.29	4.99
	19.50	18.88	14.35	5.54	4.80	4.98
	13.05	17.37	9.06	6.32	6.90	3.48
	20.19	16.08	12.85	9.92	4.40	2.97
Duroc	11.12	13.30	9.49	16.99	4.24	9.38
	55.71	8.81	8.62	13.84	18.82	7.04
	72.33	29.33	50.03	7.83	3.99	4.99
	65.40	27.77	7.97	20.39	7.83	4.28
	94.39	24.76	12.46	9.91	5.20	5.40
	30.23	11.68	28.59	17.50	11.05	
Pietrain	24.63	11.27	11.66	11.43	1.37	2.60
	5.71	21.58	8.78	2.65		1.58
	3.87		2.27	1.92	2.17	8.30
	5.16	2.65	2.22	9.26	7.67	1.44
	2.43	3.15	2.41	5.41	9.96	1.21
	4.45	2.72	2.47	1.73	7.13	5.77

– A^{pat}, – G^{pat}, – undetermined

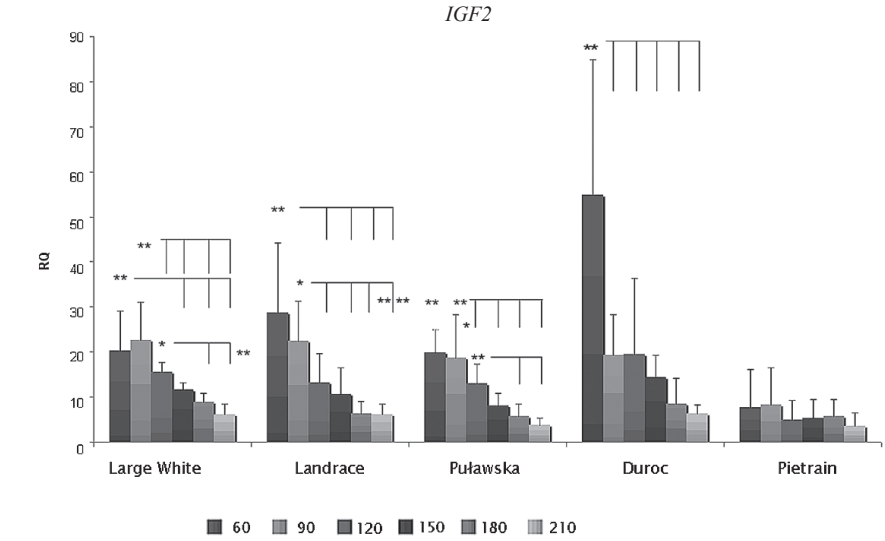

Figure 1. Relative Quantity (RQ) of *IGF2* transcript in muscles during development of pigs
* – P<0.05, ** – P<0.01.

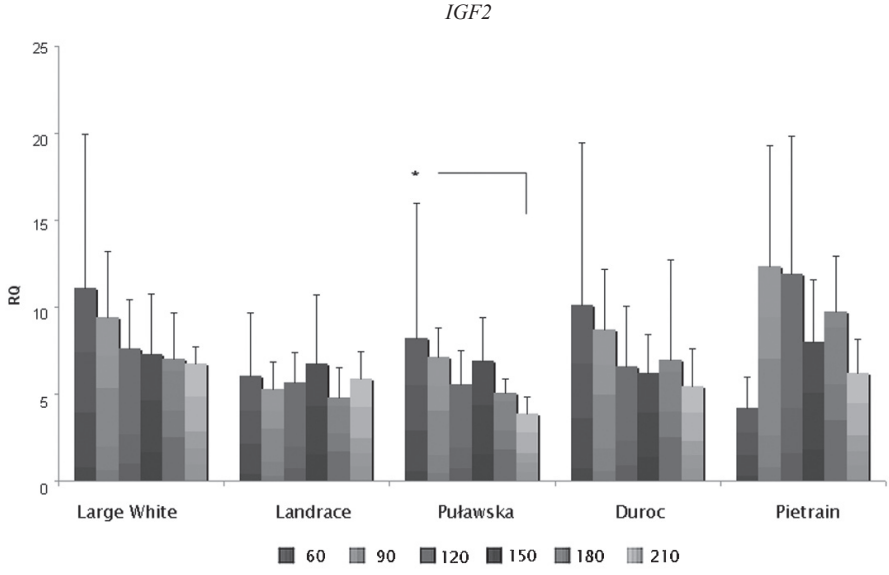

Figure 2. Relative Quantity (RQ) of *IGF1* transcript in muscles during development of pigs
* – P<0.05, ** – P<0.0.1.

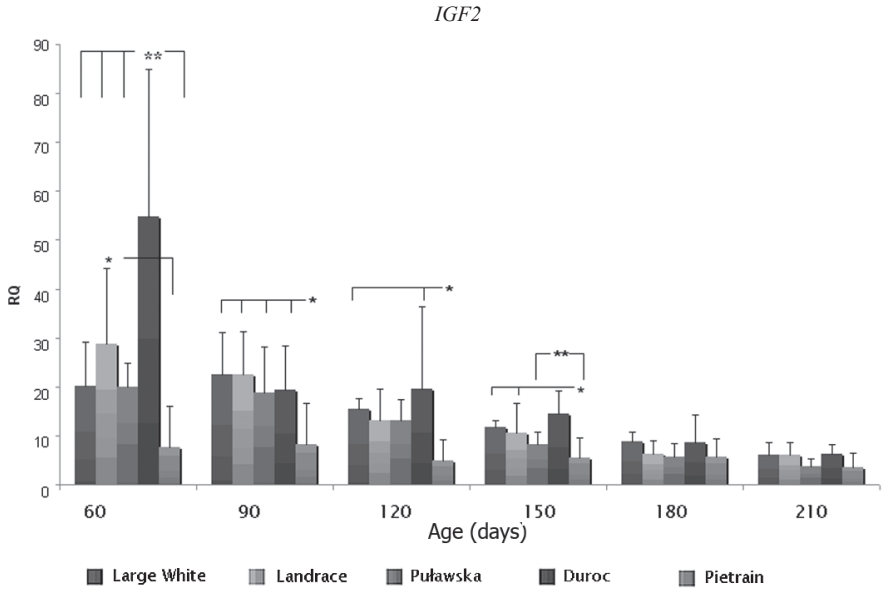

Figure 3. Relative Quantity (RQ) of *IGF2* transcript in muscles of pigs representing different breeds
*– P<0.05, ** – P<0.01.

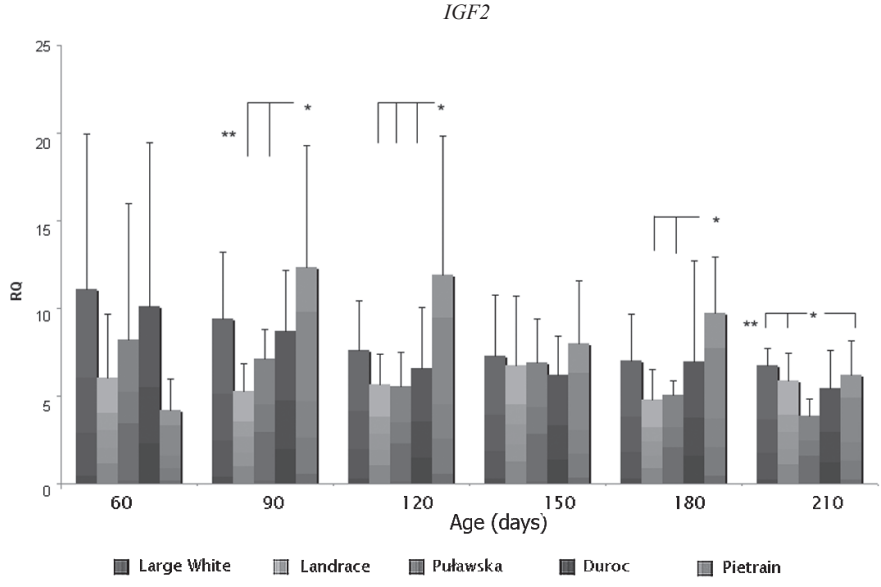

Figure 4. Relative Quantity (RQ) of *IGF1* transcript in muscles of pigs representing different breeds
* – P<0.05, ** – P<0.01.

Discussion

A decrease of *IGF1* and *IGF2* expression in muscles with the age of the pigs has been reported previously and corresponds to the fact that relative muscle growth decreases with age (Gerrard et al., 1998; Stinckens et al., 2007).

In our study, lower level of *IGF2* transcript in the Pietrain breed is the result of a large number of animals carrying paternally derived G allele in the Pietrain group (Table 1). The Pietrain group in our study also differed from other groups in respect to *RYR1* genotype (many of Pietrain animals were C/T heterozygotes).

It was proved previously that animals which inherited the G allele from the father, displayed a 3-fold lower expression of *IGF2* gene compared with A/A homozygotes or heterozygotes which inherited the G allele from the mother (Van Laere et al., 2003; Stinckens et al., 2007). Recently, an interaction between *IGF2* gene and *RYR1* gene on fibre type composition has been reported (Van den Maagdenberg et al., 2008). It was suggested that the mechanism involved in increased leanness due to the impaired *RYR1* receptor might influence the underlying mechanisms of the *IGF2* mutation. What is more, *IGF2* expression in *longissimus dorsi* depended on *RYR1* genotype – significantly higher expression was found in the stress resistant group, compared with the TT group (Stinckens et al., 2007). Unfortunately, unequal distribution of *IGF2* genotypes in our study did not allow us to statistically analyse interactions between *IGF2* and *RYR1* genotype in respect to *IGF2* expression.

On the other hand, the Duroc breed displayed the highest expression of *IGF2* gene. At the age of 60 days the expression level of *IGF2* gene in the Duroc breed was significantly higher than in all other breeds (Fig. 2). This observation is surprising as all Duroc, Large White and Landrace pigs carried paternally derived A allele, and one can assume that there is another mutation apart from G3072A, which may influence expression level of *IGF2* gene. Jungerius et al. (2004) suggested the presence of a second minor QTL affecting backfat thickness in SSC2p. Moreover, Estelle et al. (2005) demonstrated that there are QTL for carcass weight and meat quality segregating in SSCp2 other than the *IGF2* substitution.

Interestingly, Pietrain – the breed with the highest meat content, displayed the highest expression level of *IGF1* at some developmental stages. Similar results were obtained by Hoeflich et al. (2004), who examined expression patterns of the insulin-like growth system in mice selected for growth. They observed that expression of *IGF1* is higher in muscles of mice with high lean body mass, compared with mice with low lean body mass.

In conclusion, our results confirmed that the level of *IGF1* and *IGF2* expression in muscles decreases during development of animals. We observed significantly lower expression level of *IGF2* gene in the Pietrain group in which many animals carried paternally derived G allele of *IGF2* gene and many animals were C/T heterozygotes in *RYR1* gene. Our results also suggest that a new putative mutation in *IGF2* gene may influence the gene expression as animals carrying the same paternally derived allele differed in *IGF2* expression level. Further studies are needed in order to evaluate if the overexpression of *IGF1* in Pietrain has a biological meaning and to identify genetic factors involved in this higher expression.

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Ekspresja genów *IGF1* i *IGF2* w mięśniu w trakcie rozwoju świń reprezentujących pięć różnych ras

STRESZCZENIE

Insulinopodobne czynniki wzrostu są częścią osi somatotropowej – grupy substancji zaangażowanych w kontrolę wielu ważnych funkcji w organizmie ssaków, w tym rozwoju mięśni. W przeprowadzonych badaniach analizowano zmiany w poziomie ekspresji genów *IGF1* i *IGF2* w mięśniach świń w trakcie rozwoju (pomiędzy 60. a 210. dniem życia). Porównano również poziom ekspresji tych genów u świń należących do pięciu ras, różniących się cechami użytkowości. Poziom ekspresji genu *IGF2* znacznie spadał (nawet 9-krotnie) wraz ze starzeniem się zwierząt. Poziom *IGF1* również spadał, jednak w dużo mniejszym stopniu. Porównania międzyrasowe potwierdziły wpływ znanej mutacji *IGF2* G3072A SNP na poziom ekspresji *IGF2*, a także wskazały na obecność dodatkowego czynnika mogącego wpływać na poziom ekspresji tego genu. Ponadto, stwierdzono podwyższoną ekspresję genu *IGF1* w rasie Pietrain w niektórych okresach rozwojowych.