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Expression of the fibronectin gene in *longissimus dorsi* and *semimembranosus* muscles in Polish breeds of pigs*

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Fibronectin is an adhesive glycoprotein found on cell surfaces, in the intracellular and extracellular matrix and in biological fluids. It was shown that fibronectin increases level of IGFBP5 in muscles *in vitro.* What is more, fibronectin plays a crucial role in muscle oxygenation during exercise.
The aim of the study was to compare the expression of the fibronectin (FN1) gene in *longissimus dorsi* and *semimembranosus* muscles of pigs and then to determine the relationship between the level of the expression and the age of animals. Compared was also the level of FN1 expression in muscles among different pig breeds in order to establish if there are differences between commercial and primitive lines.
The results did not show a clear expression pattern of FN1 during pig development. Higher expression of FN1 in *semimembranosus* than in *longissimus dorsi* was found in all breeds at almost all developmental stages.

KEY WORDS: fibronectin / gene expression / pigs / real-time PCR

Fibronectin (FN1), an adhesive glycoprotein, is commonly found on cell surfaces, in the intracellular and extracellular matrix (fibril-forming insoluble polymer) of different types of connections or muscle tissue, and in body fluids (soluble form in blood plasma) – Wójtowicz [2006]. Fibronectin is known to influence various

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processes, from fetal morphogenesis to tissue proliferation and differentiation, and contribution to inflammatory processes as a mediator of virus infection [Liu and Collodi 2002]. It also plays a key role in the adhesion of cells to the matrix and in the mutual identification [Yamada and Clark 1996]. Although fibronectin has been studied for almost 30 years, its novel properties are still being discovered such as a finding in this protein, new integrin receptor binding sites [Mostafavi-Pour *et al*. 2001, Liao *et al*. 2002]. What is more, the discovery of fibronectin's role in wound healing and clot formation in the 1990s [Romberger 1997] has spawned research on new applications in the form of modified fibronectin preparations that accelerate the wound healing process [Ghosh 2006], especially at ischaemic sites.

The role of fibronectin in muscle oxygenation during exercise is crucial. During deformation of skeletal muscles under physical load, fibronectin molecules change in shape, resulting in emission of a signal that relaxes smooth muscles surrounding blood vessels. This change in the shape of protein molecule "exposes" the site that is normally concealed within the structure and triggers a cascade of signals, which dilates the blood vessels and increases blood flow through the muscles [Hocking 2008]. Recently, it was shown that fibronectin binds to IGFBP-5 and this binding negatively regulates the ligand-dependent action of IGFBP-5 by triggering IGFBP-5 proteolysis. Insulin-like growth factor binding protein-5 (IGFBP-5) is a secreted protein that binds to IGFs and modulates IGF actions on cell proliferation and differentiation [Xu *et al*. 2004].

The objective of this study was to compare the expression of the fibronectin gene in *longissimus dorsi* and *semimembranosus* muscles and then to determine the relationship between the level of expression and the age of the animals. Compared was also the level of FN1 expression in muscles of pigs of different breeds in order to establish if there are differences between animals representing commercial and primitive lines.

Material and methods

Samples were studied of *longissimus dorsi* and *semimembranosus* muscles, taken from Polish Large White (PLW), Polish Landrace (PL), Duroc, Pietrain and Puławska pigs. Animals were kept in 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, six groups of animals of each breed were formed (5-6 sows per group): 60-, 90-, 120-, 150-, 180- and 210-days-old. Animals were related – all sows within the breed had the same father (except the Pulawska breed – three fathers), and their mothers were sisters. Tissue fragments were collected immediately after slaughter and kept in liquid nitrogen during transportation. RNA was isolated from the samples of muscle tissues from the slaughtered animals, using the method reported by Chomczyński and Sacchi [2006].

Quality of RNA was evaluated by gel electrophoresis. Furthermore, the amount of RNA was checked spectrophotometrically to standardize the next stages of analysis.

The RNA samples were then brought to the same concentration and transcribed into cDNA *via* reverse transcription using APPLIED BIOSYSTEMS reagents (High Capacity cDNA Reverse Transcription Kit, cat. no. 4368813) according to the manufacturer's protocol.

The obtained cDNA was analysed by relative quantitation (RQ) using real-time PCR. Primers (F:5'-TCTGTTGACCAAGGCCTAAGC-3'; R:5'-GGCATAATGGGAAACCGTGT A-3') and a fluorescently labelled TaqMan probe (Fam-5'-CACGGATGACTCGTGCT TCGACCC) were designed by using Primer Express software attached to a 7500 Real-Time PCR System (APPLIED BIOSYSTEMS).The GAPDH gene (glyceraldehyde-3-phosphate dehydrogenase), which expression level in the muscles did not change considerably during the animal development, was used as endogenous control [Hoogewijs *et al*. 2008]. Real-time PCR (RQ) was performed using a series of product dilutions (separately for the analysed gene and endogenous control) to determine their yield. The similar reaction yields [Nygard *et al.* 2007] for the fibronectin and GAPDH genes made it possible to use the ΔΔCT method for analysis of the results [Yuan *et al.* 2006].

Results and discussion

The results showed no common expression pattern of FN1 during development in pig breeds considered. Expression level of FN1 during development differed also in two analysed muscles. In the *longissimus dorsi* of Puławska breed the highest expression level was observed on day 90 of age and the mean mRNA abundance was significantly different from those found on day 120, 150, 180 and 210. Moreover, expression on day

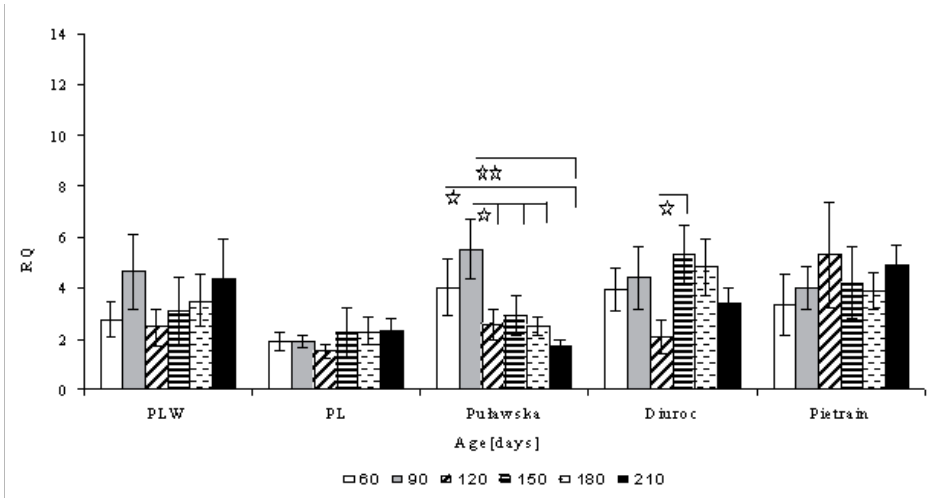

Fig. 1. Expression of the FN1 gene determined in the *longisimus dorsi* muscle; Relative Quantity (RQ). *P< 0.05, **P< 0.01.

60 was slightly lower than on day 90 of age and significantly different from that on day 210. In Durocs, expression of FN1 was similar at all developmental stages apart from day 120 of age, when the expression was the lowest and significantly different from that found on day 150. In all other breeds expression level was not related to developmental stages (Fig. 1).

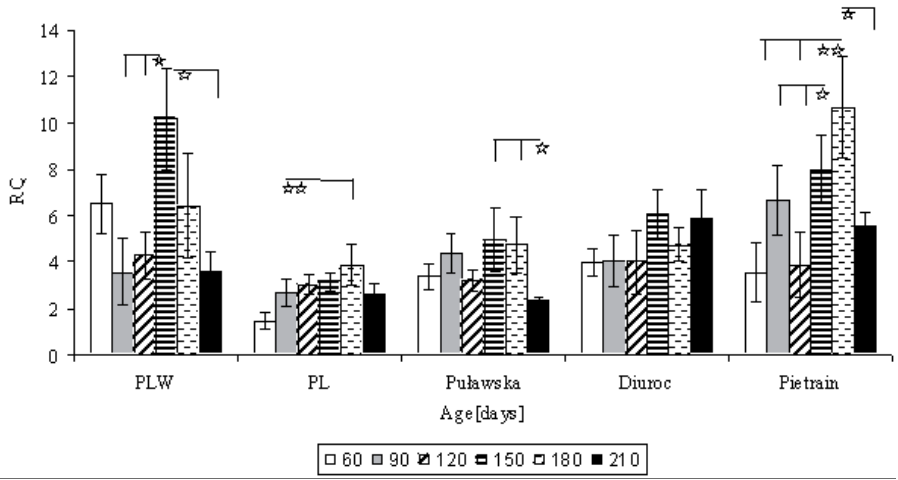

Fig. 2. Expression of the FN1 gene determined in the *semimembranosus* muscle; Relative Quantity (RQ). *P< 0.05, **P< 0.01.

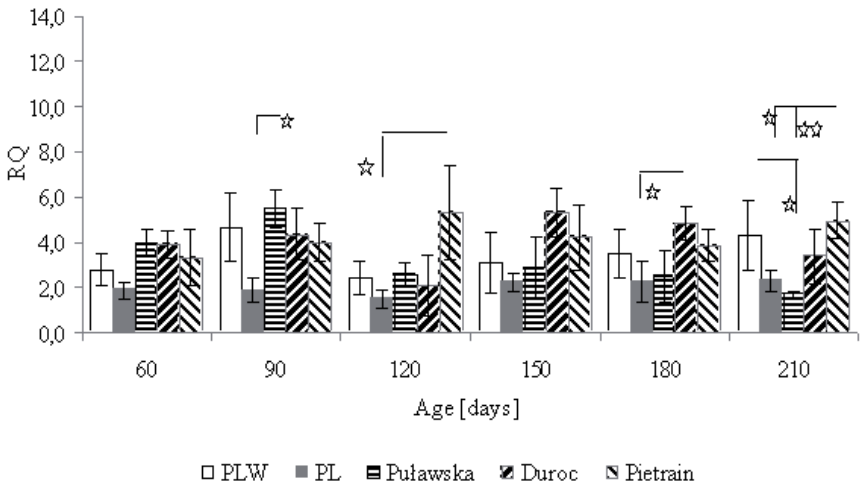

Fig. 3. Expression of the FN1 gene determined in the *longisimus dorsi* muscle; Relative Quantity (RQ). *P< 0.05, **P< 0.01.

In the *semimembranosus* muscle of Duroc pigs expression did not change significantly during development. In Pietrain and PL the highest expression level was at the age of 180 days, whereas in PLW and Puławska on day 150 of age. In Puławska breed the lowest expression occurred at the age of 210 days (Fig. 2).

FN1 mRNA abundance in *longissimus dorsi* of Pietrain breed was approximately 3-fold higher than of Puławska on day 210 of age. Expression level of FN1 seemed to be lowest in Landrace pigs at most of the developmental stages (Fig. 3).

On the other hand, FN1 expression level in *semimembranosus* differed between breeds almost at every developmental stage. The only exception was day 120, when the expression level did not differ among breeds. Similarly to the *longissimus dorsi*, the lowest expression of FN1 was in Landrace at all developmental stages. However, not all of the differences were identified as significant (Fig. 4).

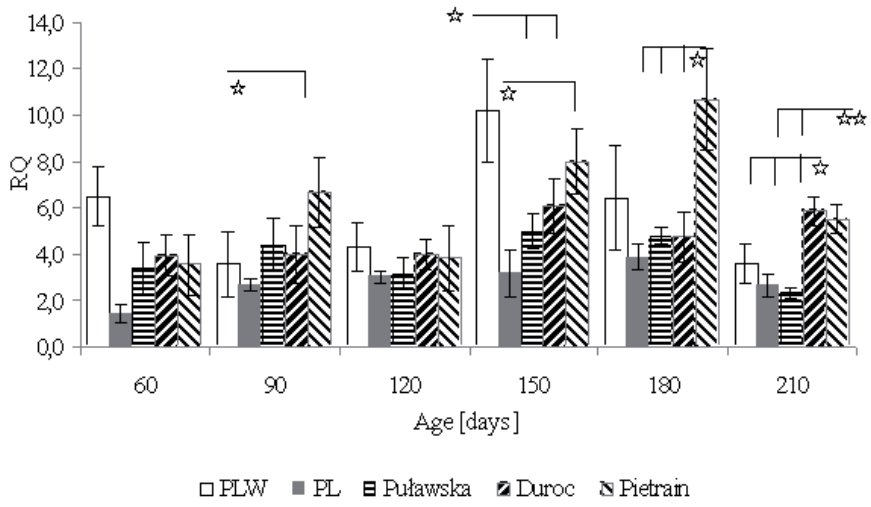

Fig. 4. Expression of the FN1 gene determined in the *semimembranosus* muscle; Relative Quantity (RQ). *P< 0.05, **P< 0.01.

Comparison of two muscle types (*longissimus dorsi* and *semimembranosus*) shows that FN1 expression level is higher in the latter (Fig. 5). Significant differences were observed on day 60 of age in Large White, on day 150 in Large White and Pietrain, and on day 180 and 210 in Durocs.

A typical for all breeds expression pattern of FN1 during development was not observed. Expression fluctuations in some breeds were most probably the result of inter-individual variation. On the other hand, higher expression of FN1 in *semimembranosus* than in *longissimus dorsi* muscle was noticeable in all breeds at almost all developmental stages. It can reflect morphological differences between

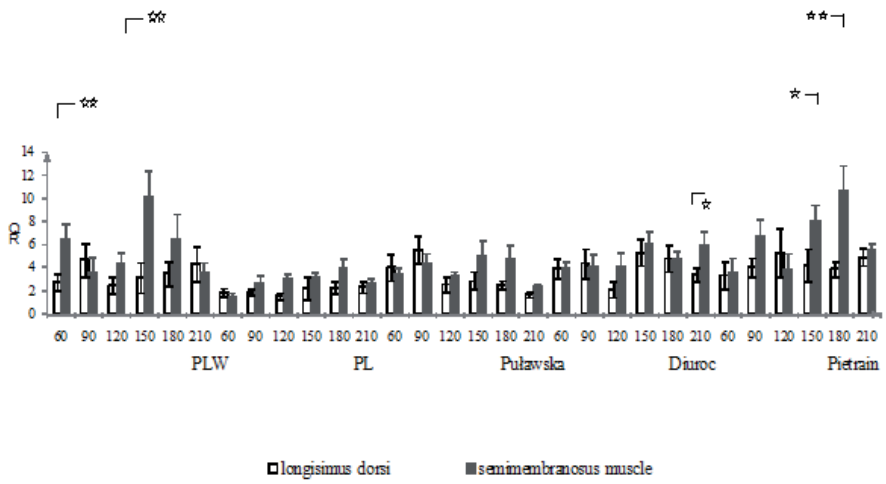

Fig. 5. Expression of the FN1 gene determined in the *longisimus dorsi* and *semimembranosus* muscles; Relative Quantity (RQ). *P< 0.05, **P< 0.01.

the two muscle types. Further studies are needed in order to recognize whether the differences in FN1 mRNA abundance observed in Landrace, comparing with other breeds have any biological significance.

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Ekspresja genu fibronektyny w mięśniach najdłuższym grzbietu i półbłoniastym świń utrzymywanych w Polsce

S t r e s z c z e n i e

Fibronektyna jest adhezyjną glikoproteiną obecną na powierzchni komórek, a także w macierzy wewnątrz- i zewnątrzkomórkowej oraz w płynach ustrojowych. Niezwykle ważną jej cechą jest udział w dotlenieniu mięśni podczas wysiłku. W badaniach in vitro wykazano, że zwiększa ona poziom IGFBP5.

Celem pracy było oznaczenie ekspresji genu fibronektyny w mięśniach najdłuższym grzbietu i półbłoniastym szynki, a następnie określenie zależności między jej poziomem, a wiekiem i rasą świń.

Stwierdzono wyższą ekspresję w mięśniu półbłoniastym niż najdłuższym grzbietu we wszystkich badanych rasach, jednak nie można na tej podstawie jednoznacznie określić profilu ekspresji w trakcie rozwoju.