MUSCLE PROTEIN PROFILES OF TRANSGENIC PIGS EXPRESSING A BOVINE GROWTH GENE

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S-VII.02

SUMMARY

Protein profiles of the longissimus muscles (LM) from transgenic pigs, expressing a bGH gene, and control pigs were investigated. Each transgenic (T) pig was compared with a control (C) pigs of similar body-weight. Samples were excised within 1 h post mortem. Myofibrillar and sarcoplasmic proteins were separated and the protein profile was assessed by means of SDS-PAGE (7.5, 12 and 15% polyacrylamide). Neither molecular weight nor concentration of the various proteins differed between C- and T-pigs. Both the myofibrillar and sarcoplasmic protein profiles of C- and T-pigs were similar.

Introduction

During the past decade, scientists have learned how to transfer genes into animals of different species, creating the so-called 'transgenic' animals. The application of new genetic manipulation techniques offers tremendous potential for enhancing carcass composition. Transgenic pigs expressing a bovine growth hormone gene (T-pigs) are considerably leaner (as much as 85%) than control (C) pigs at the same weight and age (Solomon et al. 1992).

In 1988, Solomon and Dunn developed a combined myofibrillar (acid) ATPase and succinic dehydrogenase staining procedure and determined that minor procedural modifications were necessary when examining muscle from different species. Interestingly, when this procedure was applied to muscle of T-pigs, the bovine-version of the staining procedure was necessary in order to achieve effective fiber type differentiation (Solomon et al., 1991). This observation suggested genetically determined differences in the muscle composition of T-pigs. Therefore, we decided to investigate the protein profile of the longissimus muscle (LM) from T- and C-pigs.

Materials and methods

Fifty-two transgenic and sibling, control pigs were used. Details on production and treatment of the animals are described by Solomon et al. (1992). Each T-pig was compared with a C-pig of similar body-weight. Within 1 h after slaughter, samples (ca. 1x1x3 cm) of the longissimus muscle (LM), 13th rib location, were excised and immediately restrained on flat sticks. Muscle samples were frozen in liquid nitrogen and stored at -70°C until further analysis.

A 2 gram portion of muscle was removed from each frozen sample and immersed in 10 volumes of the 'extraction' buffer (75mM KCl, 5 mM KPO_4, 2mM EGTA, 2 mM $MgCl_2$, pH=7.2). After the sample had thawed, it was cut in small pieces and 'incubated' in the same buffer for at least 1 h at 0-4°C. Subsequently, the samples were homogenized and the homogenate centrifuged (15 min, 1000xg, 4°C). The supernatant was used for analysis of sarcoplasmic proteins. The pellet was resuspended in the extraction buffer, homogenized and centrifuged. This wash procedure was repeated 4 times. After the last wash, myofibrillar fractions were resuspended in 0.0625 M Tris, pH 6.8, and protein concentration was assessed using the biuret procedure (Gornall et al. 1949). The myofibrillar suspensions were diluted to a concentration of 4 mg/ml and diluted (1:1) with sample buffer (0.0625 M Tris, pH 6.8, 20% glycerol, 10% ß-mercaptoethanol, 4% SDS and 0.001% bromophenol blue).

After assessment of protein concentration of the sarcoplasmic fraction, these were diluted to 4 mg/ml with the extraction buffer and mixed (1:1) with sample buffer.

After addition of sample buffer, all the samples were heated at 90°C for at least 10 min before being stored at -20°C.

Before analysis samples were thawed and reheated for at least 5 min. In both myofibrillar and sarcoplasmic samples the protein profile was assessed by means of SDS-PAGE (7.5, 12 and 15% polyacrylamide). SDS-PAGE was performed by a modified Laemmli (1970) method, using a 4.5% acrylamide stacking gel and a 7.5, 12 or 15% polyacrylamide separating gel. The gels were stained with Coomassie Brilliant Blue R.

Results and Discussion

The myofibrillar protein profiles are shown in Fig. 1 through 3 and the sarcoplasmic profiles are shown in Fig. 4. Although different concentrations of acrylamide were used in an attempt to magnify differences in proteins profiles, few differences were evident. In general, neither molecular weight nor concentration of the various proteins differed for C- and T-pigs. Myofibrillar and sarcoplasmic protein profiles of C- and T-pigs were similar. The small inconsistent differences observed in creatine kinase and phosphorylase bands were probably due to variation in stress before and during slaughter. Stress could result in increased denaturation of these sarcoplasmic proteins, causing them to precipitate out on myofibrillar proteins.

Solomon et al. (1991) observed a significant difference in fiber type distribution between T- and C-pigs; the T-pigs having fewer red and more intermediate fibers. According to Young and Davey (1981), fiber types can be characterized by myofibrillar protein composition; the relative migration (=molecular weight) of heavy chain myosin, tropomyosin, troponins I and C and myosin light chains of fast and slow fibers was shown to be different. We did not observe any consistent differences in these proteins between T-pigs and C-pigs. Possibly, the differences reported by Solomon et al. (1991) were too small to be detected using electrophoresis. Also, the study of Young and Davey (1981) concerned bovine muscle.

It may be concluded that there are no significant differences in myofibrillar and sarcoplasmic protein profiles of T-pigs vs. C-pigs. It is not clear what exactly causes the muscle from T-pigs to respond like bovine muscle when subjected to the combined staining procedure. It may be that differences in proteins are too small to be detected by SDS-PAGE. Alternatively, it may be that the observed differences do not reflect a difference in concentration or molecular weight, but rather a difference in amino acid composition. Currently, additional characterization of proteins expressed by T-pigs is underway, using procedures such as iso-electric focussing.

References

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Legends to Figures 1-4:

Figure 1-3: SDS-Page of myofibrillar proteins from longissimus muscle of transgenic (T)-pigs and control (C)-pigs. Weight indicates body weight of pigs.

Figure 1: 7.5% polyacrylamide;

Figure 2: 12% acrylamide;

Figure 3: 15% acrylamide.

Figure 4: SDS-Page of sarcoplasmic proteins from longissimus muscle of transgenic (T)-pigs and control (C)-pigs; 15% polyacrylamide. Weight indicates body weight of pigs.

Explanation of symbols:
a= myosin heavy chain; b= α-actinin; c= phosphorylase B; d= actin; e= creatine kinase; f= troponin T; g= α-ß tropomyosin; h= myosin light chain I; i= troponin I and C; j= myosin light chain II; k=pyruvate kinase; l=enolase; m=aldolase; n=myoglobin;
M= molecular weight marker containing: phosphorylase (94000 daltons), serum albumin (67000 daltons), ovalbumin (43000 daltons), carbonic anhydrase (30000 daltons), trypsin inhibitor (20100 daltons) and α-lactalbumin (14400 daltons).