BETA-ADRENERGIC FEEDING DIFFERENTIALLY ALTERS FIBRE TYPE-SPECIFIC GENE EXPRESSION IN PORCINE MUSCLE

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Introduction

The primary goal of animal agriculture is to produce the greatest quantity of high quality protein possible for the least amount of input. Much progress has been made over the past few decades, mainly as a result of improved nutrition and genetics, and from the use of growth promotants in meat-animals. However, as incremental increases in muscle protein deposition become more difficult to achieve through traditional means, we must delve further into the biological mechanisms controlling muscle growth. One aspect of muscle biology that has been largely overlooked by most in the area of enhancing muscle growth is by understanding how muscle-specific contractile genes differentially respond to hypertrophy signals. Although understandable, many scientists have erroneously assumed that when muscle experiences hypertrophy all contractile proteins are up-regulated. However, muscle consists of a heterogeneous population of muscle cells (fibres), which collectively dictates the overall biochemical and contractile nature of muscle. Depending on the physiological status of the animal, (i.e., growing, maintaining, senescing, etc.) muscle has the ability to modify its functional characteristics to accommodate these 'states'. Molecular mechanisms controlling this phenomenon are unknown, but are particularly intriguing to those interested in animal agriculture because the abundance of each type of fibre in muscle are associated with changes in animal performance and ultimate meat quality development. One commercially used swine growth promotant, Paylean®, dramatically increases the amount of type IIB myosin heavy chain (MyHC) in skeletal muscle at the expense of slower-specific MyHC isoforms (Depreux *et al.*, 2002). The active ingredient in Paylean®, ractopamine, is a beta-adrenergic agonist that repartitions nutrients in growing pigs toward muscle and increases protein accretion (Beermann, *et al.*, 1987). Ultimately, the feeding of such a growth promotant results in animals that grow faster and more efficient, and yield carcasses with more muscle and less fat. The exact mechanisms underlying this improved efficiency are not well understood but may be a result of differentially changing muscle fibre type-specific gene expression. Therefore, the objective of this experiment was to determine the relative expression of muscle fibre type-specific genes after feeding ractopamine.

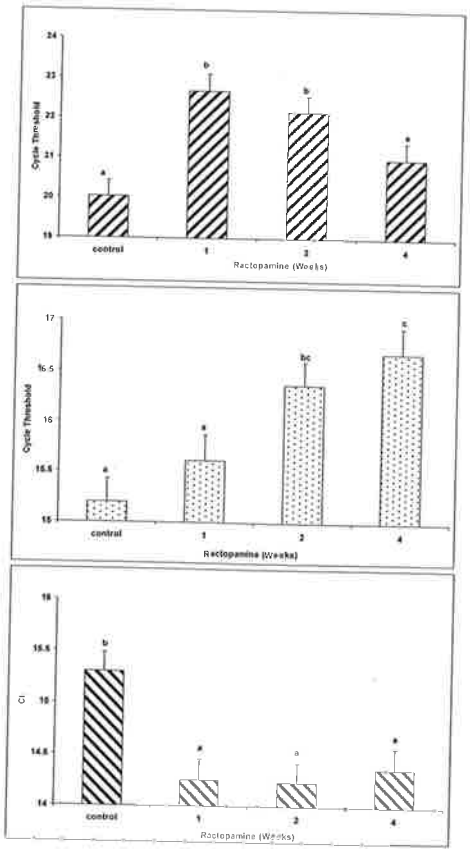
Materials and Methods

Ractopamine (RAC, Paylean®, Elanco Animal Health, IN) was administered at 20 mg/kg daily in feed for either 0, 1, 2 or 4 wk to 44 pigs (90 kg) obtained from Purdue University Animal Sciences Research and Education Center. Control pigs were slaughtered after a 4 wk period of growth, whereas pigs that were fed RAC for 1 or 2 wk followed a 3 or 2 wk period of growth, respectively. This was an attempt to achieve the predetermined duration of feeding, and target an average slaughter weight of 115 kg. Pigs were processed according to normal industry procedures and LD muscle samples were taken immediately post-exsanguination. Samples were frozen in liquid nitrogen and stored at -80°C. Total RNA was extracted and quantified using the Ribogreen quantification kit (Molecular Probes, Eugene, OR). RNA was reverse transcribed and subjected to real time polymerase chain reaction (qPCR). Primer sequences for myosin heavy chain isoforms I, IIA, IIX, and IIB, GS, CS, and β-actin have been described previously (de Costa, *et al.*, 2002). Additional primer sequences for PPARα, and β-ARs 1 and 2 were used as well. The first cycle in the log linear region of amplification where a significant increase in fluorescence was detected above background was designated the threshold cycle (Ct). Therefore, a low Ct indicates high gene expression. Data were analyzed using the GLM procedure of SAS with Ct as the independent variable. Data were reported as least square means and an analysis of variance was used to determine differences among those means.

Results

Real time PCR was used to determine the effect of RAC on gene expression in porcine skeletal muscle. The control to test for experimental variation, β-actin, did not differ over the course of the experiment. Expression of the type I MyHC gene was not altered by RAC during the entire length of the study (data not shown). Type IIA MyHC gene expression was decreased ($P < 0.0001$) 7-fold by 1 wk of RAC administration, remained 4-fold lower ($P < 0.001$) at 2 wk, but was not different from controls by 4 wk (Figure 1). RAC did not influence MyHC type IIX gene expression by 1 wk, but decreased ($P < 0.001$) expression by 2 and 4 wk (Figure 1). MyHC type IIB expression was increased ($P < 0.0001$) 2-fold by 1 wk, and remained elevated throughout the remainder of the study (Figure 1). Citrate synthase (CS) gene expression was not different from controls at 1 wk, but decreased ($P < 0.01$) by 2 and 4 wk (data not shown). Glycogen synthase (GS) mRNA abundance was greater ($P < 0.05$) by 1 wk of RAC administration, returned to the level of control at wk 2, and decreased ($P < 0.01$) by 4 wk (data not shown). Lactate dehydrogenase (LDH) gene expression was not affected by RAC. Expression of the PPARα gene decreased ($P < 0.05$) by 1 wk of RAC administration, but was not

different from controls by 2 and 4 wk. RAC had no effect on β_1-AR gene expression (data not shown), however, β_2-AR gene expression decreased ($P < 0.05$) by 2 and 4 wk (data not shown).



Discussion

Although MyHC genes are homologous, expression of each isoform was affected differently by RAC in our study. Furthermore, changes in gene expression reported in our study mirrors RAC-induced changes in fibre type frequency as well as MyHC protein abundance (Aalhus *et al.*, 1992; Depreux *et al.*, 2002). Therefore, RAC-induced muscle hypertrophy is likely a result of transcriptional regulation of MyHC genes. Effects of RAC administration over a 4 wk period are shown in Figure 1 and, although the effect on type IIA MyHC gene expression returns to the level of controls at wk 4, we found no attenuation of MyHC type IIX and IIB gene expression, suggesting our study concluded before RAC-induced signal transduction was attenuated. Together, results presented herein provide evidence indicating RAC differentially affects MyHC gene expression, resulting in an acute transition between MyHC isoforms (1 wk), and suggest a chronic adaptation of energy metabolism (4 wk) is likely after prolonged stimulation by a β-agonist.

Conclusions

Data presented herein suggest RAC differentially induces expression of type II MyHC genes. However, RAC was administered to pigs during the finishing phase where pigs normally exhibit muscle hypertrophy. Control pigs in the current study experienced muscle growth, therefore, it cannot be concluded that RAC causes muscle hypertrophy. The possibility remains that muscle fibre type-specific gene expression was altered because muscle was growing more rapidly. Further research is needed to determine if RAC alters gene expression in porcine skeletal muscle in the absence of hypertrophy to more clearly define a cause-and-effect relationship between altered gene expression and muscle hypertrophy.

Figure 1: Differential expression of type IIA (top), IIX (middle) and IIB (bottom) myosin heavy chain isoform in pig longissimus muscle. Pigs were fed 20 ppm ractopamine for 0 (control), 1, 2 and 4 wks prior to slaughter. Muscle samples were taken and subjected to real time PCR analysis to determine relative MyHC gene expression. Note: Cycle threshold is inversely related to mRNA abundance, as lower amounts of mRNA require more cycles to cross the detection threshold of the detector.

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