A Further Look at the Effects of Growth Hormone on Morphological Muscle Characteristics in Pigs

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SUMMARY

The effects of porcine growth hormone (pGH) on longissimus muscle morphological characteristics were different than those of bovine growth hormone (transgenic, T-pigs) in pigs. Classical porcine fiber arrangement was less evident in T-pigs compared to pGH treated pigs. The distribution of muscle fiber types was altered in T-pigs, yet not affected by pGH administration. Red fibers tended to be larger in T-pigs while I and W fibers were smaller. On the contrary, all three fiber types increased in size as a result of pGH administration. T-pigs displayed no visual signs of pale, soft, exudative (PSE) muscle while a 33% incidence of PSE was observed for pGH treated pigs.

INTRODUCTION

Animal growth is a complex, physiological process regulated by the endocrine system, which in turn mediates the effects of nutritional, environmental and genetic factors in animals. Meat animals, (e.g., pigs) have been intensively selected for maximal growth rate.

With greater emphasis on lean tissue deposition and less lipid in meat producing carcasses, several studies in our laboratory were conducted with designs to address the question of defining genetic potential for protein deposition in the pig and the resulting effects on muscle morphology and meat quality. This paper compares two current strategies to increase meat yield and reduce fat which have been evaluated in our laboratory [(1) administration of exogenous porcine growth hormone (pGH) to pigs and (2) transgenic pigs expressing a bovine growth hormone gene (T-pigs)].

Growth hormone is generally believed to be the most important hormone affecting growth and development. The technology for introducing foreign genes into laboratory animals has been available only since 1980 (reviewed by Palmiter and Brinster, 1986). Introducing foreign genes into mammalian embryos forms the basis of a powerful approach for studying gene regulation and the genetic basis of biological development. For the most part, the mouse has been the choice of experimental animal. Extension of gene transfer into other species is required to evaluate the potential for improving production efficiency and disease resistance of domestic livestock. Successful application of gene transfer techniques to farm animals has been reported (Hammer et al., 1985, 1986; Pursel et al., 1987, 1989).

Exogenous porcine growth hormone (pGH) administration markedly improves growth performance and body composition of pigs at different stages of growth (Machlin, 1972; Etherton et al., 1987; Campbell et al., 1988, 1989). Solomon et al., (1988) demonstrated that pGH administration to young growing pigs (from 25 to 55 kg) resulted in an increase in muscle fiber mass with no effect on muscle fiber distribution. Similarly a 31-d pGH administration to pigs with an initial live weight of 60 kg (Solomon et al., 1991) resulted in an increase in muscle fiber size with no effect on fiber distribution. In these studies pGH administration resulted in hypertrophied (giant) fibers. The occurrence of giant muscle fibers in porcine skeletal muscle has been linked with stress-susceptibility in pigs which exhibit pale, soft, exudative (PSE) muscle (Mircheva and Vitanov, 1987).

MATERIALS AND METHODS

Animals. Six transgenic pigs produced by the microinjection of single-cell zygotes and two-cell ova with linear molecules of mouse metallothionein I promoter/regulator fused to bovine growth hormone (bGH) structural gene (described in detail Pursel et al., 1987) were compared to six littermate controls. In a separate study, twelve crossbred pigs with an initial live wt of 36 kg were injected daily with pGH (dose levels 0 and 100 ug/kg/d) for a 33-d period. These pigs were fed a corn-soybean meal, skim milk based diet containing 18% protein and 35 Mcal DE/kg.

Histochemical. Samples (1x1x3 cm) of the longissimus (LM) muscle (13th rib location) from the right side of each carcass were obtained within 1 h postmortem and immediately restrained on flat sticks. Length of muscles was measured prior to excision in order to maintain proper fiber length when restrained on the flat sticks. Muscle samples subsequently were frozen in liquid nitrogen. Frozen samples were stored at -70°C until histochemical analyses were performed. A 1-cm^3 fragment of tissue removed from each frozen sample was mounted on a cryostat chuck with a few drops of water so that muscle fiber orientation was perpendicular to the cutting blade of the microtome (Damon Minotome™ Microtome-Cryostat, Needham Heights, MA). Mounted samples were allowed to equilibrate to -20°C. Sections (12 um thick) were cut with the microtome. Sections were treated with the combination myofibrillar (acid) ATPase and succinic dehydrogenase staining procedure described for porcine skeletal muscle by Solomon and Dunn (1988).

The stained slides were observed with a Zeiss Standard #16 photomicroscope (Carl Zeiss, Inc., New York, NY). Fibers were classified according to Ashmore and Doerr (1971) on the basis of stain reaction (R, I, W). All fibers inside a template (46.7 cm^2 in area) were counted and measured for cross-sectional area using a Zeiss Interactive Digital Analysis System (Carl Zeiss, Inc., New York, NY) as described by Solomon and Montgomery (1988).

Data Analysis. Data were analyzed by the analysis of variance technique (SAS, 1985) to determine the significance of variation between the different treatments.

RESULTS AND DISCUSSION

Longissimus muscle samples from T-pigs and their control counterparts were treated with the porcine combination myofibrillar (acid) ATPase and succinic dehydrogenase staining procedure described by Solomon and Dunn (1988). The porcine combination stain was effective at differentiating muscle fiber types in the control pigs, yet unsuccessful for the T-pigs. After several unsuccessful attempts (for T-pigs) with the porcine combination stain, the bovine combination stain procedure (described by Solomon and Dunn, 1988) was used and successful fiber differentiation was accomplished. All three fiber types were present at varying proportions in the T-pigs. The distribution and areas of muscle fiber types in the LM as affected by bGH (T-pigs) are shown in Table 1. The effect of bGH was limited to the percentage of R and I fibers. T-pigs had fewer R fibers (42%) and more (21%) I fibers than the controls.

The classical porcine fiber arrangement (R fibers grouped in clusters surrounded by I and W fibers) was less evident for the T-pigs than the controls. Hypertrophied (giant) fibers were not present in either the T-pigs or their control counterparts. The occurrence of giant muscle fibers has been associated with stress-susceptible pigs, which exhibit pale, soft, exudative (PSE) muscle. Neither the T-pigs nor the control pigs displayed any visual indication of PSE muscle, even though T-pigs exhibited multiple signs of stress-sensitivity (Pursel, 1990 personal communications). Red fibers tended to be larger in T-pigs while I and W

fibers were smaller in T-pigs compared to controls. At present no literature is available to compare morphological and meat quality characteristics for T-pigs expressing a bovine growth hormone gene, therefore, we will make comparisons with the pGH treated pigs.

Successful muscle fiber type stain was accomplished for pGH treated pigs using the porcine combination stain reaction (Solomon and Dunn, 1988). The classical porcine fiber arrangement was clearly evident for both pGH treated and non-treated pigs. This is in contrast to what was observed with the T-pigs. The distribution and areas of muscle fiber types in the LM as affected by pGH administration are presented in Table 2. The administration of pGH had no effect on the percentage distribution of muscle fiber types. We previously reported (Solomon et al., 1988, 1991) that pGH did not alter muscle fiber type profiles (percentages) in the LM muscle of pigs regardless of time of administration or age of the pigs being treated. Beerman et al. (1987) examining the relationship of pGH dose response to skeletal muscle growth, also found that pGH did not affect fiber type percentages in the semitendinosus muscles of pigs slaughtered at 100 kg live wt. On the contrary, bGH in the T-pigs (Table 1) altered the fiber population, in particular R and I fibers. Ashmore et al. (1972) evaluated porcine muscles histochemically to determine growth patterns and concluded that I fibers have the capacity to transform into W fibers. Transformation is concerned primarily with changes in energy-producing enzymes and is accompanied by a rapid increase in fiber size. Furthermore, they suggested that muscle size was directly proportional to the degree to which I fibers transform into W fibers. The differences observed for T-pigs does not appear to be related to the energy-producing enzymes transformation described by Ashmore et al. (1972) but related more to compositional differences associated with species differences (bovine vs porcine).

Porcine growth hormone increased the areas of all three fiber types by an average of 22% in the LM. The greatest increase in size was observed for W fibers (24%) followed by a 20% increase for I fibers and 17% increase for R fibers. These findings are in agreement with those by Beerman et al., (1987) and Solomon et al. (1988, 1991), who reported similar increases in fiber size for all three fiber types with the administration of pGH. In comparing T-pigs with pGH treated pigs, we see that R fibers were the only fibers to increase in size as a result of growth hormone (bGH-T pigs) administration. The I and W fibers were, in fact, substantially smaller in the T-pigs than the controls. Hypertrophied (giant) fibers were present in all of the LM samples from the pGH treated pigs, however, none were present in those pigs used as controls for the PGH treatment study. Nor were they present in the LM of the T-pigs.

Two of the six pGH treated pigs exhibited PSE muscle which represents a 33% incidence of PSE as a result of pGH administration. Hypertrophy of muscle fibers seems to be a direct action of pST administration and is not necessarily associated with the PSE syndrome (Solomon et al., 1991). In previous studies (Solomon and West, 1985; Solomon and Eastridge, 1987; Solomon et al., 1988, 1990, 1991) we either observed no PSE syndrome with hypertrophied fibers or minimal PSE with hypertrophied fibers. A seasonal effect was proposed (Solomon et al., 1991) as one possible explanation for the inconsistent incidence of PSE with pGH administration. The occurrence of PSE in conjunction with pGH administration could also be due to genetic differences between the pigs used in the different studies.

CONCLUSION

The effects of pGH on porcine LM muscle morphological characteristics were different than those of bGH - transgenic pigs. The distribution of muscle fiber types was altered in T-pigs and is probably a result of genetic-species differences in muscle compositional characteristics. Typical porcine muscle morphology was

less evident in T-pigs. Therefore, the major influence of introducing a foreign gene (bGH) into pigs on muscle characteristics seems to be within species specificity. The major influence pGH had on muscle was a hypertrophic effect.

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TABLE 1. COMPARISON OF LONGISSIMUS MUSCLE FIBER[a] DISTRIBUTION AND AREAS FOR TRANSGENIC[b] AND CONTROL PIGS

	Fiber type, %			Fiber area, um^2		
Item	R	I	W	R	I	W
Transgenic	7.2[d]	24.2[c]	68.6	2493.8[c]	1875.8[d]	2338.2[d]
Control	12.4[c]	20.0[d]	67.6	2153.3[d]	2209.7[c]	4359.3[c]

[a]Muscle fibers classified according to Ashmore and Doerr (1971).
[b]Transgenic pigs = pigs expressing bovine growth hormone gene.
[c,d]Means in the same column with different superscripts differ (P<.05).

TABLE 2. COMPARISON OF LONGISSIMUS MUSCLE FIBER[a] DISTRIBUTION AND AREAS FOR PIGS TREATED WITH OR WITHOUT PORCINE GROWTH HORMONE

	Fiber type, %			Fiber area, um^2		
Item	R	I	W	R	I	W
pGH, ug/kg/d						
0	13.8	27.4	58.8	2276.7[c]	2430.8[c]	3347.3[c]
100	12.3	27.4	60.3	2666.2[b]	2922.2[b]	4145.3[b]

[a]Muscle fibers classified according to Ashmore and Doerr (1971).
[b,c]Means in the same column with different superscripts differ (P<.05).