Muscle Growth, Development, and Meat Quality

Analysis of Myosin Heavy Chain Isoforms in Callipyge Sheep

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Skeletal muscle hypertrophy in callipyge lambs is associated with an increased percentage of fast-twitch glycolytic muscle fibers. Several muscle hypertrophy conditions exhibit changes in myosin heavy chain (MHC) isoforms. The objective of this study was to separate and quantify myosin heavy chain isoforms in muscles of callipyge and non-callipyge lambs. Longissimus muscle samples were collected from four lamb genotypes (NN, CN, NC, and CC; C represents the mutant callipyge allele and paternal allele is listed first). Muscle samples were homogenized in 10 volumes of 75 mM KCl, 10 mM KH_2PO_4, 2 mM $MgCl_2$, 2 mM EGTA, pH 7.0, containing 50 mM NaF, 2 mM phenylmethylsulfonyl fluoride, and 6 mg/L leupeptin. Homogenates were centrifuged at 10,000g for 15 minutes at 4°C to separate myofibrillar and sarcoplasmic proteins. Myofibrillar protein pellets were washed 3X in extraction buffer containing 1% triton x-100. Proteins were solubilized by heating in SDS sample buffer. Protein concentration was determined by BCA assay and samples were suspended in SDS reducing buffer containing 20% glycerol. Proteins (1 microgram/lane) were loaded onto discontinuous 8% gels (50:1 ratio of acrylamide to N,N'-methylene-bis-acrylamide) with 4% stacking gels (50:1). Gels (20 cm x 20 cm x 0.75 mm) were run at 275 V for 24 hours at 4 C using a BIO-RAD Protean II xi cell and a two buffer system. The upper running buffer consisted of 0.1 M Tris, 150 mM glycine, and 0.1% SDS. The lower running buffer contained 50 mM Tris, 75 mM glycine, and 0.05% SDS. MHC isoforms separated by SDS-PAGE were silver stained. Four MHC isoforms were separated and classified as described for rodent MHC's. Hypertrophy in phenotypic callipyge longissimus muscle (CN genotype) is associated with a transition from type IIA to type IIX MHC when compared to other lamb genotypes.

Response of Porcine Skeletal Muscle Enhanced by an IGF-I Transgene

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Two groups of sibling pigs, control (C) and transgenic (T), each with 7 barrows and 6 gilts were used to determine effects of insulin-like growth factor-I (IGF-I) specifically targeted to skeletal muscle. Transgenic pigs were produced with a fusion gene composed of avian skeletal μ-actin regulatory sequences and the cDNA encoding IGF-I. After slaughter (120 kg live wt), five muscles were dissected and weighed. Dual-energy x-ray absorptiometry detected 30% larger loin eye area ($P < .001$), 10.5% more lean ($P < .01$) and 20% less fat ($P < .01$) in the T-pigs than C-pigs for both sexes. Weights of rectus femoris (RF), psoas major (PM) and triceps brachii (TB) muscles were 19.7, 18.5, and 10.6%, respectively, heavier in T than C-pigs. Biceps femoris (BF) and semitendinosus (ST) muscles were heaviest (21.4 and 17.9%, respectively) in T-barrows compared to C-barrows, while no difference between C- and T-gilts was observed. Percentage distribution of slow-twitch oxidative (SO) and fast-twitch oxidative/glycolytic (FOG) fibers in the RF were lowest in T-barrows and highest in C-barrows but reversed for FG fibers. T-barrows had larger (3587 vs 2571 μm^2) FOG fibers than C-barrows. T-pigs had larger SO and fast-twitch glycolytic (FG) than C-pigs. No differences in fiber population were found for the longissimus (LM) muscle, all three fiber types were larger in T-pigs than in C-pigs. Targeting IGF-I for skeletal muscle growth resulted in increased carcass lean verified by 8 to 20% increase in muscle weights and 11 to 31% increase in muscle fiber areas.

Identification of Genes Differentially Expressed During Pig Skeletal Muscle Development

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The establishment of specialized cell types during development of specific tissues involves the expression of distinct sets of cell type-specific genes. We have recently initiated a project to identify genes differentially expressed during pig skeletal muscle development. Muscle tissue was obtained from the hind limbs of pigs at 60, 75, 90, and 105 d of gestation and at birth, 21, and 49 d of age. Preliminary northern blot analyses were performed for the genes myogenin (MYOG) and myogenic factor 6 (MYF6). Relative mRNA abundance of MYOG decreased from 60d of gestation to 49 d of age, whereas MYF6 mRNA abundance increased. Since patterns of MYOG and MYF6 expression were similar to those reported for other species, these samples provide a valuable resource for identification of genes differentially expressed during pig skeletal muscle development. Currently, we are using the differential display reverse transcription polymerase chain reaction (DDRT-PCR) technique to accomplish our objective. To date, we have used 5 primer pairs to identify putative differentially expressed genes in muscle tissue from 60 and 105 d of gestation and 49 d of age (3 samples per age). Our first round of screening included excision of 6 bands that were amplified in all 3 samples of at least one age and were undetectable in all samples of the remaining age(s). DNA sequence analysis of subclones derived from 2 of the bands each yielded 2 distinct gene products, whereas a single product was obtained from subclones of each of the remaining bands. Pig complementary DNAs were identified with homologies to titin, nebulin, perinatal myosin heavy chain, acidic calponin, collagen type I alpha 2, Janus kinase 1, cytochrome c oxidase subunit III, and NADH dehydrogenase subunit 4. Confirmation of differential expression of these genes, as well as cloning and sequencing of additional differentially expressed cDNAs, is currently underway. Our results indicate that DDRT-PCR is a powerful technique for identifying genes expressed in developing pig skeletal muscle.

Adding Value to Low-Quality Beef Muscles through Glycolytic Inhibition in Pre-rigor Muscle

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Pre-rigor *Semimembranosus*, *Triceps brachii* and *Supraspinatus* muscles were removed from ten steers to evaluate the effect on pH, tenderness, and color of several glycolysis-inhibiting compounds as a means to enhance value of low-quality beef muscles. Muscles were injected and tumbled with 10% (wt/wt) of one of four different solutions: sodium citrate (NaC; 200 mM), sodium fluoride (NaF; 200 mM), sodium acetate (NaA; 200 mM), and calcium chloride ($CaCl_2$; 300 mM). Control samples remained in the carcass at 2°C for 24 h. Samples were taken 3 d postmortem. Injections with NaC and NaF increased ($P < .05$) pH values in *Semimembranosus* (5.81 and 6.00, respectively) compared to control (5.24). *Triceps brachii* treated with NaC and NaF had higher pH (5.73 and 5.92, respectively) than control (5.28). Also *Supraspinatus* showed higher ($P < .05$) pH with these treatments (5.86 and 6.18, respectively) than control (5.45). Shear forces were lower ($P < .05$) in *Triceps brachii* with $CaCl_2$ (4.85 kg), NaF (5.18 kg) and NaC (4.30 kg) than controls (5.67 kg). *Supraspinatus* treated with $CaCl_2$ and NaC had lower ($P < .05$) shear force values (5.49 and 5.06 kg, respectively) than controls (6.74 kg). Treated muscles showed lower sarcomere lengths than their controls ($P < .05$). These data show high pH favors tenderness. Water holding capacity was not affected by treatments ($P > .05$). Color L* and b* values were not different among treatments ($P > 0.05$), however, treated *Semimembranosus* and *Triceps brachii* muscles had less red color than controls ($P < .05$). Sodium citrate and sodium fluoride were successful in improving beef tenderness, without detriment to lean color, by maintaining a high pH in pre-rigor muscles.

Traditional and Non-Traditional Approaches for Defining Meat Tenderness: A Regional Effort.

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As the callipyge (CLPG) phenotype could have a positive economic impact on the American lamb industry, mechanisms through which the CLPG gene is expressed has been the research focus of many scientists. CLPG gene expression produces specific muscular hypertrophy in longissimus dorsi (LD), and leg muscles, increase in feed efficiency and decrease in fatness of the carcass. Expression of this gene also results in decreased tenderness of the LD. Joint efforts by faculty from the University of Idaho and Washington State University have been conducted to define aspects of CLPG gene expression with a focus towards increasing meat tenderness and quality of CLPG influenced muscles. Long term aging improved tenderness of normal and CLPG loin chops, yet CLPG chops never reached the threshold for tenderness as normal chops aged to 80 d. Expression of the CLPG gene increased toughness of the LD with no change in tenderness of semimembranosus (SM) muscle. Freezing for at least 8 d prior to aging accelerated post-mortem tenderization in CLPG LD, without reducing juiciness or flavor. Muscle accretion rates were similar between phenotypes at 7 kg live weight, but were higher for CLPG LD and SM from individuals between 20 to 69 kg. Calpastatin activity was higher in LD and SM of CLPG, while Warner-Bratzler shear force differed between phenotypes in LD only. While satellite cells isolated from CLPG animals

possessed higher growth rates than similar cells from aged-matched (normal) counterparts, responsiveness of all cells to extrinsic regulatory factors is still being defined. Further, a satellite cell and adipocyte co-culture system is being devised with one goal to define intercellular regulation differences normal vs CLPG cells. These data demonstrate that a comprehensive, northwest effort has been established to define mechanisms underlying meat tenderness and quality of CLPG lambs.

Selection for Lean Growth Efficiency in Duroc Pigs: Impact on Pork Quality.

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The objectives of the project were 1) to characterize the impact of selection for lean growth efficiency on fresh pork quality and 2) define biological factors associated with deterioration of pork quality in pork from pigs selected for lean growth efficiency. Selection over five generations has generated a line of Duroc pigs (S) with greater lean growth efficiency when compared to a randomly selected contemporary control (C) line. Carcass and quality data were collected from barrow carcasses from each litter in generation 5 (n=39). All pigs in the study tested negative for the halothane gene. Lean gain was positively affected by the selection strategy. Select line carcasses had greater percent muscle, less average backfat, and 10th rib fat. Select line loin chops had higher percent protein, lower percent lipid, lower firmness scores, and higher Warner-Bratzler shear values than C-line chops. Longissimus dorsi, semimembranosus, and semitendinosus chops from S-line carcasses had greater moisture and protein lost as drip than C-line chops. Selection did not affect longissiums dorsi calpastatin activity or muscle fiber type determined by SDS-PAGE. Select line chops did demonstrate a slower production of a 30 kDa product identified by an anti-troponin-T antibody. There was no selection line effect on glycolytic potential in longissimus dorsi samples taken at 15 minutes postmortem. However, the longissimus dorsi from the select line had a significantly higher amount of lactic acid and a lower pH at 15 minutes than the control line. Selection for some economically important traits such as feed efficiency or increased lean growth in the absence of the Halothane gene may compromise pork quality. Pinpointing the causative agents involved in these processes will aid in developing selection strategies that will efficiently produce lean, high quality pork.

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Effects of Short Term Feeding of Vitamin D_3 on Pork Quality

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Two preliminary experiments and one main experiment were conducted to determine if dietary vitamin D_3 could be used to improve tenderness of pork. On the basis of the two preliminary experiments a dose of 500,000 IU/d of vitamin D_3 fed for 3 days was used for the main experiment. In the main experiment twenty-four market weight barrows were fed a control diet or a diet supplemented with 500,000 IU/d of vitamin D_3 for 3 days. Initial plasma calcium concentrations were not different, but the pigs supplemented with the vitamin D_3 for 3 days had a higher plasma calcium concentration just prior to slaughter ($P < .01$). Although there were no differences in beginning and ending body weights, the pigs fed the control diet tended to gain more weight ($P < .12$). Also, the controls tended to also have a greater dressing percentage ($P < .10$). There were no differences in hot carcass weight ($P < .21$), but control fed pigs had a heavier 24-hour carcass weight ($P < .05$). Vitamin D-fed pigs tended to have a higher 24-hour carcass shrinkage ($P < .20$) and a greater ultimate longissimus pH ($P < .04$). However, other 24-hour carcass quality measurements showed no differences between treatments. Vitamin D supplementation also tended to increase water holding capacity of loins at day 1 ($P < .12$). However, yield and purge of hams were unaffected. Both the results of Warner-Bratzler shear and Star probe measures of tenderness of chops of the longissimus muscle showed no differences. Thus, our hypothesis that supplemental dietary vitamin D_3 given prior to slaughter would improve pork tenderness was not supported by this study.

Prediction of Pork Carcass Composition For Potential Use in Market Reporting: Compositional Variation in Carcasses Classified as US 1

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Pork carcasses (n = 133), varying in fatness and muscling, were used to document the compositional variation present in the US 1 slaughter hog grade and the inability of the US slaughter hog grading system to accurately segregate carcasses into distinct compositional classes. Carcasses, from commercial packing plants, were selected in a 3 x 3 factorial arrangement with three levels of 10^{th} rib fat depth (BF; < 2.03, 2.03 to 2.54, and > 2.54 cm) and three levels of loin eye area (LEA; < 35.5, 35.5 - 41.9, and > 41.9 cm^2). Carcass data was collected on ribbed carcasses which were fabricated to the following endpoints: percent boneless, denuded cuts (BDC),

percent fat free lean (FFL), and total fat (TF) percent. Data were analyzed using a least squares fixed effects model. Prediction equations, using maximum R^2 procedures, were developed for the compositional endpoints using carcass measurements. Fatness and muscling traits increased significantly as BF and LEA category increased, respectively. Compositionally, BDC and FFL decreased and TF increased incrementally with increasing BF level. Although carcasses in the two leanest BF categories would be classified as US 1, a 3.5, 5.4, and 7.4% difference in BDC, FFL, and TF, respectively, existed within those carcasses. As LEA increased, FFL decreased; however, BDC and TF were only significantly different in the smallest LEA category. Finally, BDC, FFL, and TF could be accurately predicted (R^2 = .76, .78, and .87, respectively) using BF and other measures of carcass muscling, with BF accounting for 65, 70, and 81% of the variation, respectively. Collectively, these data show the significant impact of carcass muscling and fatness on pork composition and the need to revise the slaughter hog grading system to accurately segregate pork carcasses based on value differences. Furthermore, it appears that compositional endpoints can be accurately predicted using easily estimated measures of carcass fat and muscling.