Influence of genotype, sex, and dietary lysine on carcass quality characteristics of 104 and 127 kg finishing pigs. S.R. Stuewe*, J.A. uh, K.G. Friesen, J.L. Nelssen, R.D. Goodband, and M.D. Tokach, Kansas e University, Manhattan.

otal of 116 growing/finishing pigs (fed from 44 to 104 or 127 kg) were zed to determine the interrelationships among genotype, sex, and dietary ne on carcass quality characteristics. The experiment was designed in a 2 × 2 factorial arrangement and analyzed as a randomized complete block. Pigs e derived from genotypes previously characterized based upon lean gain enial (high vs medium lean gain; HLG and MLG, respectively). Within otype, pigs were split by sex (barrows vs gilts) and fed one of two dietary ne treatments. From 44 kg to a pen average of 104 kg, pigs were fed either 0 or .70% lysine corn-soybean meal diet. When the average pen weight ched 104 kg, one pig was slaughtered, and the remaining two pigs were fed s reduced from .90 or .70% to .75 or .55% dietary lysine, respectively. At en mean of 127 kg, the remaining two pigs were slaughtered. After 24 h mortem, 10th rib longissimus muscle (LM) quality traits were measured and chops were frozen until further analysis. For carcasses from pigs fed to 104 LM from HLG had ($P<.05$) less visual marbling and higher Hunter a* ire red), Hunter b* (more yellow), and higher saturation index (more vivid ntense) color values than LM from MLG. In a genotype × lysine interaction $<.05$), HLG pigs fed .70% lysine and MLG fed .90% lysine had higher $<.05$) LM pH's than MLG pigs fed .70% lysine. However, all pH means e in the normal range of 5.4 to 5.5. In a genotype × sex interaction $<.05$), HLG gilts had ($P<.05$) smaller LM Hue angles (more red on a red to nge scale) than MLG barrows. Cooking losses were greater ($P<.05$) and rner-Bratzler shear (WBS) values were higher ($P<.05$, tougher) for chops n HLG gilts than HLG barrows. For LM from pigs fed to 127 kg, HLG e visually more reddish pink and firmer, but had less marbling than MLG $<.05$). Also, LM from HLG had ($P<.05$) a higher pH, less moisture date, lower Hunter L* values (darker) and smaller Hue angles than LM from G. Barrows had ($P<.05$) more LM marbling and less chop thaw loss than s. In a genotype × sex interaction ($P<.05$), HLG barrows had ($P<.05$) ner chop WBS values than HLG gilts, MLG barrows, and MLG gilts. For s fed to either 104 or 127 kg, dietary lysine had minimal effects on LM lity traits and HLG pigs had less LM marbling than MLG pigs. However, influence of genotype and sex for other LM quality traits varied between pigs to either 104 or 127 kg.

ey Words: Pork Quality, Genotype, Lysine

87 Preparation of highly homogeneous muscle talin and ts interaction with other actin-membrane attachment site roteins. J. M. Schmidt* and R. M. Robson, Iowa State niversity, Ames.

ne of our long-term goals is to understand how the eripheral layer of myofibrils in skeletal muscle cells and he contractile apparatus of smooth muscles cells are onnected to the muscle cell membrane. Talin (~230 kDa) is ne of the proteins that is located at actin-membrane ttachment sites in both muscle cell types. Our objectives n this study were to prepare highly homogeneous talin and to haracterize interactions between talin and other actin-embrane attachment site proteins. The procedure developed or isolation of talin involved the following steps: (1) xtraction of trimmed, ground muscle (gizzard) twice with ater, (2) overnight extraction of the muscle residue at 4°C n a high ionic strength buffer containing protease nhibitors, (3) ammonium sulfate fractionation, and (4) uccessive chromatography on DEAE Sepharose, hydroxyl-patite, and phosphocellulose columns. Talin-actin nteractions have been examined by using a cosedimentation ssay and by low shear viscometry. Known amounts of omogeneous talin were added to tubes of G-actin, the actin as polymerized by addition of 2 mM $MgCl_2$, incubated for 1 hr t 25°C, and centrifuged at 100,000 x g for 20 min to ediment F-actin and any bound protein. Examination of the upernatants and sediments by sodium dodecylsulfate-olyacrylamide gel electrophoresis indicated that talin nteracts with actin. Analysis of talin-actin mixtures by ow shear viscometry (falling ball) also indicated an nteraction between these proteins. The effect of other roteins, such as tropomyosin and vinculin, on the talin-ctin interaction is being examined. This study indicates hat talin interacts with actin and may play an important ole in linking the contractile apparatus to the cell embrane of muscle cells.

ey Words: Talin, Muscle cell cytoskeleton, Actin attachment sites

86 Effect of ß-adrenergic agonist ($L_{644,969}$) on calpastatin messenger RNA expression in beef and sheep longissimus dorsi muscle. J. Killefer*, M. Koohmaraie, and T. L. Wheeler. USDA/ARS, Meat Animal Research Center, Clay Center, NE 68933.

The objective of this study was to determine the effects of the ß-adrenergic agonist (BAA) $L_{644,969}$ (Merck, Sharp and Dohme Research Laboratories) on calpastatin mRNA expression levels in control and treated beef and sheep longissimus dorsi (LD) muscle. Samples were obtained immediately upon slaughter from four control and four treated (3 ppm BAA) MARC III composite steers and eight control and eight treated (4 ppm BAA) wether lambs. Total RNA was extracted from a one gram sample of each muscle and subjected to slot blot and Northern blot analysis using a new cDNA probe homologous to bovine skeletal muscle calpastatin. Autoradiographs were analyzed by densitometric analysis. Total calpastatin mRNA was determined from the linear portion of individual slot blot RNA standard curves (0.1 - 3.2 µg/slot) and normalized to total calpastatin/µg total RNA. BAA treatment was shown to increase the total calpastatin mRNA ($P < .01$) by greater than two-fold in both beef and sheep which is in agreement with calpastatin enzyme activity data as previously reported. Northern blot analysis revealed that skeletal muscle calpastatin mRNA is present as three transcript sizes (3.8, 3.0 and 1.5 kbp) in beef and as four transcript sizes (3.8, 3.0, 2.5 and 1.5 kbp) in sheep. Densitometric analysis of the Northern blots revealed that the 3.8 and 3.0 kbp transcripts are the most responsive to BAA treatment with the 3.8 kbp form decreasing and the 3.0 kbp form increasing significantly from the controls ($P < .01$). The ratio of the 3.0 kbp band to the 3.8 kbp band has a positive correlation with LD Warner-Bratzler shear values at 14 d postmortem (beef, $r = .66$; sheep, $r = .69$). These results indicate that calpastatin mRNA and enzyme activity levels increase in a coupled fashion with BAA treatment, suggesting that regulation of calpastatin activity is at the transcriptional level under these conditions. Selective responsiveness of the multiple calpastatin mRNA transcript forms suggested that BAA-sensitive mechanisms were controlling exon splicing and/or selection of polyadenylation sites which in turn controlled the stability and cellular function of calpastatin.

Key Words: Calpastatin, Expression, Beta-adrenergic agonist

88 Evidence that the 30 kDa polypeptide in postmortem skeletal muscle comes from troponin-T. C.-Y. Ho*, M. H. Stromer, and R. M. Robson. Iowa State University, Ames.

A 30 kDa polypeptide is frequently seen by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of postmortem aged muscle samples. The presence and amount of this 30 kDa polypeptide is sometimes used as an indication of the rate and extent of postmortem proteolysis. It has been hypothesized that the 30 kDa band originates from degradation of troponin-T (TN-T). We have used antibodies to various myofibrillar proteins to identify this polypeptide. One side of 10 beef carcasses was electrically stimulated (ES) after slaughter with 250 volts, 20 Hz for 45 sec to achieve a 3 hr postmortem pH value of 5.9-6.3. The other side served as the non-stimulated (NS) control. The longissimus muscle was sampled immediately after stimulation and at 1, 3, 7, 14 and 28 days postmortem. A 5 cm cross section of the LD muscle posterior from the 13th rib was removed from the carcass at 1 day postmortem and was used for subsequent samples. All samples were stored at 2°C. SDS-PAGE and Western blots were used to monitor postmortem changes and to identify specific proteins. In SDS-PAGE of NS samples, the TN-T band decreased in density by 7 days and disappeared by 14 days. In ES samples, TN-T was absent by 7 days and indicated that a more rapid degradation was occurring. In SDS-PAGE, a trace amount of a 30 kDa band was seen in the 1 day NS sample but a larger amount of a 30 kDa band was seen in 1 day ES sample. In blots, a monoclonal antibody (mAb) to TN-T labeled both TN-T and the 30 kDa polypeptide. The prominent 30 kDa band that showed a large increase in density in the 1 day ES samples was consistently labeled by the TN-T mAb. Antibodies to other myofibrillar proteins did not label the 30 kDa band. Although small amounts of TN-T could still be detected in blots after 14 and 28 days in both ES and NS samples, amounts were much less in ES samples. Based on these results, we conclude that ES caused more rapid degradation of TN-T and earlier appearance of the 30 kDa polypeptide in bovine longissimus muscle. Labeling with a mAb to TN-T positively identifies the 30 kDa polypeptide as a degradation product of TN-T.

Key Words: TN-T, 30 kDa polypeptide, skeletal muscle, SDS-PAGE