

Muscle and Lipid Biology and Biochemistry

132: AGING-INDUCED CHANGES IN SARCOPLASMIC PROTEOME OF THREE BEEF HINDQUARTER MUSCLES WITH DIFFERENTIAL COLOR STABILITY

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Objectives: Fresh beef color is critical to consumers' purchase decisions. Beef color stability is muscle-specific, and the muscle-specific variations in sarcoplasmic proteome influence beef color. Post-mortem aging is a common practice employed by beef industry for improving beef tenderness and palatability. However, the color attributes and sarcoplasmic proteome of beef muscles undergo changes during aging. The objective of this study was to examine the changes in the sarcoplasmic proteome profile of three differentially color-stable muscles from beef hindquarters during postmortem aging.

Materials and Methods: Longissimus lumborum (LL), psoas major (PM), and semitendinosus (ST) muscles were obtained from both sides of eight (n = 8) beef carcasses (USDA Choice, 24 h post-mortem). Muscles were further divided into two equal-length sections and vacuum-packaged. The vacuum-packaged muscle sections were randomly assigned to aging at 2°C for either 0, 7, 14, or 21 days. On each aging period, muscle sections were fabricated into 2.5-cm thick steaks, individually over-wrapped, and allocated to refrigerated storage for 0, 3, or 6 days. Samples for proteome analysis obtained during fabrication were frozen at -80°C. On each storage day, lightness (L^*), redness (a^*), yellowness (b^*), hue (trueness of red), chroma (saturation index), pH, and metmyoglobin reducing activity (MRA) were evaluated. The instrumental color, pH, and MRA data were analyzed using MIXED procedure in SAS. Sarcoplasmic proteome was analyzed using two-dimensional electrophoresis (pH 5–8; 13.5% acrylamide gels). The images of Coomassie Blue-stained gels were obtained and analyzed. Protein spots exhibiting 1.5-fold intensity difference ($P < 0.05$) were considered differentially abundant and were subjected to tryptic digestion and tandem mass spectrometry for identification

Results: The results indicated that instrumental color attributes and biochemical parameters during storage were influenced by muscle source and aging ($P < 0.05$). LL and ST had greater ($P < 0.05$) surface redness (a^* value) than PM, whereas the color stability (R630/580) followed the order: LL > ST > PM. Aging also influenced surface redness with 7-day aged steaks demonstrating greatest values ($P < 0.05$). Proteome analysis identified differentially abundant glycolytic enzymes between the treatments (muscle source and aging days; $P < 0.05$) indicating muscle-specific changes in sarcoplasmic proteins during aging. The glycolytic enzymes identified (triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, enolase, and phosphoglucumutase-1) were more abundant ($P < 0.05$) in color-stable LL and ST compared to color-labile PM.

Conclusion: Our results indicated that the color attributes and sarcoplasmic proteome profile of beef LL, PM, and ST were influenced by aging for 21 days. Furthermore, the aging-induced changes in the sarcoplasmic proteome profile and color traits were muscle-specific. The differentially abundant glycolytic enzymes could be used as biomarkers for beef color, and for developing muscle-specific processing strategies to improve beef color stability.

Keywords: Beef color, Muscle-specificity, Proteome, Wet-aging

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133: RACTOPAMINE INFLUENCES MUSCLE PROTEOME PROFILE OF POSTMORTEM BEEF LONGISSIMUS LUMBORUM

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Objectives: Ractopamine is a beta-adrenergic agonist approved for use in cattle and pigs as a repartitioning agent to increase muscle deposition and potentially limit fat deposition. While the effects of ractopamine on proteome profile of postmortem pork muscles have been examined recently, its influence on beef muscle proteome has not been evaluated. Therefore, the objective of this study was to examine the effects of ractopamine on muscle proteome of postmortem longissimus lumborum (LL) from beef cattle.

Materials and Methods: Crossbred steers housed in pens were fed either a corn-based basal diet (CON) or a diet top-dressed with Optaflexx 45 (Elanco Animal Health) to provide 400 mg of ractopamine hydrochloride/steer per day (RAC). Ractopamine was fed the last 28 days before slaughter. Steers were harvested, and carcasses were chilled. The LL muscle samples were obtained from the carcasses of nine (n = 9) RAC and CON steers 24 h postmortem. The muscle samples were individually vacuum-packaged and frozen at -80°C for proteome analysis. Whole-muscle proteome was analyzed using two-dimensional electrophoresis, and the digital gel images were analyzed. The protein spots exhibiting more than 1.5-fold intensity differences ($P < 0.10$) between RAC and CON were subjected to in-gel tryptic digestion and were identified by tandem mass spectrometry.

Results: Five differentially abundant protein spots identified were of greater ($P < 0.10$) abundance in LL samples from RAC compared to those from CON. The proteins identified were F-actin-capping protein subunit $\beta 2$, PDZ and LIM domain protein-3, heat shock protein β -1, myoglobin, and L-lactate dehydrogenase A chain. The differentially abundant proteins belong to four functional groups; i.e., skeletal muscle organization (F-actin-capping protein subunit $\beta 2$, and PDZ and LIM domain protein-3), chaperone activity (heat shock protein β -1), oxygen transportation (myoglobin), and energy metabolism (L-lactate dehydrogenase A chain). The over-abundance of F-actin-capping protein subunit $\beta 2$ as well as PDZ and LIM domain protein-3 in RAC may be attributed to the increase in myofibrillar protein synthesis and increase in muscle mass as a result of ractopamine feeding. Heat shock protein β -1 is a chaperone that protects muscle proteins, and its increased abundance in RAC compared to CON may be due to the increased muscle protein synthesis. The over-abundance of myoglobin could possibly result from the increased oxygen consumption due to additional muscle mass accretion in RAC compared to CON, whereas the increased levels of L-lactate dehydrogenase A chain in RAC could potentially be due to the shift of muscle fiber type.

Conclusion: The findings indicated that feeding ractopamine to steers influences the abundance of proteins involved in skeletal muscle organization, chaperone activity, oxygen transportation, and energy metabolism in postmortem beef LL muscle.

Keywords: Longissimus lumborum, Muscle proteome, Ractopamine

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134: EFFECT OF OIL SOURCE, COOKING METHOD, AND STORAGE TIME ON FATTY ACID COMPOSITION IN GROUND BEEF PATTIES FROM NELLORE CATTLE

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Objectives: The aim of this study was to evaluate the effects of feeding vegetable oil sources (sunflower - SU; linseed - LO and soybean - SO) on fatty acids composition of raw and cooked beef patties stored for 0 and 90 d.

Materials and Methods: Ninety-six Nellore steers were fed diets containing 3.5% vegetable oils (DM basis). After 82 d on feed, animals were harvested and samples of Longissimus muscle and subcutaneous fat were collected to prepare hamburger patties (n=40 per treatment; 100g patty). Patties were prepared utilizing a commercial formulation (85.4% meat, 12% fat, 2% salt, 0.3% garlic and 0.3% emulsifier) and packaged in oxygen permeable plastic bags, then immediately frozen at -18°C and stored for 0 (fresh) and 90d. The patties were evaluated raw and cooked. The cooked patties were grilled at 170°C for 4 min on each side (internal temperature 70°C). Fatty acid composition was estimated using gas chromatography. The data was analyzed as a completely randomized design in a 4 × 2 × 2 factorial arrangement (3 oil sources plus control × 2 storage time × raw and cooked) using a mixed model (MIXED procedure of SAS), including the fixed effects of oil source, storage time, cooking method, and the interaction between the treatments where each patty was used as an experimental unit.

Results: There was an interaction between storage times, cooking methods and oil source (P<0.0001). There was a higher concentration of SFA for SU and SO compared to the other treatments for fresh raw samples (P<0.0001). The cooking process decreased SFA concentration of all the treatments with oil (P<0.0001). There was a higher concentration of SFA for the control samples compared to the treatments with oil source for cooked patties (P<0.0001). Both raw and cooked patties had a higher concentration of MUFA in the control treatment (P<0.0001). Cooking process did not affect MUFA concentration in any treatment (P=0.19). There was higher concentration of PUFA of LO, followed by SO (P<0.0001), while SU and control samples did not differ (P=0.16) for fresh raw patties. Cooked samples with oil had higher concentrations of PUFA than the control (P<0.0001). The cooking process did not affect the concentration of PUFA for control and LO, however it increased for SU (P<0.0001) and SO (P=0.0004). The raw patties had a higher PUFA:SFA ratio for LO treatments, whereas the cooked patties had a higher ratio for all the oil source treatments compared to control. There was an increase in the PUFA:SFA ratio after cooking for SU (P<0.0001) and SO (P<0.0001), whereas neither control (P=0.27) nor LO (P=0.38) were affected. Comparing the effect of storage time, the samples that were not stored had higher concentration of PUFA in LO especially for 18:3 n3, and SO for 18:2 n6, followed by SU and control. There was a decrease in the PUFA concentration when the patties were stored for 90 d for all the treatments (P<0.0001). The LO patties had a higher concentration of PUFA compared with patties from the other treatments (P<0.0001) stored for 90 d.

Conclusion: In conclusion, ground beef patties made from the LO and SO had higher concentrations in PUFA, the cooking process decreased the SFA for patties with oil and the storage for 90 d decreases the concentrations of PUFA.

Keywords: Bos indicus, linoleic acid, Linseed oil, vegetable oil

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135: EFFECTS OF POSTMORTEM AGING ON SMALL HEAT SHOCK PROTEIN DEGRADATION OF THREE BOVINE MUSCLES

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Objectives: Meat tenderness is a result of enzymatic degradation of muscle structural proteins. Small heat shock proteins (HSPs) are a family of molecular chaperones that could be involved in postmortem meat tenderization through its protective role in programmed cell death, namely anti-apoptotic activity. The rate and extent of proteolytic enzyme activity (particularly calpain1) change and its subsequent impacts on myofibrillar protein degradation during aging have been well established. However, the impact of postmortem aging on small HSP degradation of different beef muscles has not been fully understood. Therefore, the objective of this study was to determine the effect of postmortem aging on small HSP dynamics and its relevance to meat quality attributes of different beef muscles.

Materials and Methods: At 1 day postmortem, three muscles (*longissimus lumborum* (LL), *semimembranosus* (SM), and *psaos major* (PM)) from 8 beef carcasses were divided into 5 sections, vacuum-packaged, and assigned for 1, 2, 9, 16, and 23 days of aging. Warner-Bratzler shear force (WBSF) and water-holding capacity (WHC) including drip loss, cook loss, and purge loss were determined at each aging point. Western blots were performed to determine the extent of myofibrillar protein degradation (desmin and troponin-T), calpain1 autolysis, and small HSPs including HSP27, HSP20, and $\alpha\beta$ -crystallin intact/degradation. The experimental design was a split-plot design with muscle effect as whole plot and aging time as sub-plot. The data were analyzed by using PROC MIXED procedure of SAS. Spearman ranking correlations between protein dynamics and meat quality attributes were analyzed by using PROC CORR of SAS.

Results: Postmortem aging improved WHC of beef muscles indicated by decreased cook loss and drip loss of beef samples ($P < 0.05$). Shear force values decreased with aging as expected ($P < 0.05$). However, the different aging response for tenderness development was observed in a muscle specific manner, where PM exhibited the most rapid WBSF decrease, followed by LL and SM ($P < 0.05$). A significant decrease in intact desmin and troponin T along with increased degradation products of these proteins were found with prolonged aging ($P < 0.05$). Desmin and troponin T degradation were positively correlated with WHC, tenderness and degradation of HSP20, HSP27 and $\alpha\beta$ -crystallin ($P < 0.05$). HSP20 and $\alpha\beta$ -crystallin exhibited similar dynamics, where significant decreases of intact proteins were observed during aging ($P < 0.05$). An increase in HSP27 degradation product of all beef muscles was found with aging in general ($P < 0.05$), but LL showed the most degradation products, while PM showed the least HSP27 degradation ($P < 0.05$).

Conclusion: The result of the current study suggests that small HSP degradation of beef muscles increase with aging, but the extent of degradation would be different in a muscle-specific manner. A different degradation pattern of HSP in LL compared to PM could be coincided with greater myofibrillar degradation of LL compared to that of PM during aging. The increase in small HSP degradation could indicate loss of protective anti-apoptotic activity from delaying myofibrillar protein degradation. Further investigation of upper stream (mitochondrial) apoptotic factors and caspase system dynamics over aging and their relationship with HSP and meat tenderness development would be warranted.

Keywords: Muscle type, Postmortem aging, Small heat shock protein, Tenderness

Muscle and Lipid Biology and Biochemistry

136: GENE CO-EXPRESSION NETWORK ANALYSIS ASSOCIATED WITH CARCASS TRAITS IN NELLORE STEERS

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Objectives: Carcass traits are influenced by a complex network of gene interactions in muscle, so elucidating the relationships between genes and how these genes influence these traits is crucial for understanding the muscle development in animals. This study aimed to identify groups of co-expressed genes in the skeletal muscle of Nelore steers associated with ribeye area (REA) and backfat thickness (BFT), using RNA-Sequencing (RNA-Seq) data.

Materials and Methods: Three hundred and ninety Nelore steers from the Brazilian Agricultural Research Corporation (EMBRAPA/Brazil), were raised in feedlots under identical nutrition and management conditions until slaughter at an average age of 25 months. Samples from *Longissimus dorsi* (LD) muscle located between the 12th and 13th ribs were collected in two time-points: at slaughter for RNA sequencing analysis to guarantee the RNA integrity, and 24 h after slaughtering for REA and BFT evaluations. A total of 43 animals were selected based on their extreme (highest (H) or lowest (L)) GEBVs (Genomic Estimated Breeding Values) for REA and BFT to define groups for differential expression analysis. RNA-Seq data was normalized by the Transcript per million (TPM) procedure. The gene co-expression network analyses was carried out using the “blockwiseModules” function, part of the WGCNA (Weighted Correlation Network Analysis) R package. Modules were merged based on the dissimilarity between their eigengenes, which is the first principal component of each module. For each module, a different color was assigned. Module-trait associations were estimated using the correlation between the eigengene module and the phenotype (REA and BFT). Genes from modules with significant module-trait associations ($P < 0.1$), for at least one trait, were assigned for functional enrichment analysis when their Module Membership (MM) values were greater than 0.7 ($P < 0.001$). The functional enrichment analysis was performed by DAVID v6.7 (FDR <0.1).

Results: Thirty-seven modules were identified. Among the modules identified, the Blue ($r=0.3$), Dark Green ($r=0.3$) and Salmon ($r=0.3$) presented significant correlation ($P < 0.10$) with BFT. The Blue module was the largest one, presenting 953 genes (MM >0.7). The functional enrichment analysis of the 953 genes from the Blue module identified 101 Gene Ontology (GO) terms including biological processes, cellular component, and molecular function, and six KEGG pathways. Among the metabolic pathways identified for the Blue module, the Extracellular Matrix - receptor interaction (bta04512) was noteworthy. This pathway was related to GO terms such as proteinaceous extracellular matrix (GO:0005578), extracellular matrix (GO:0031012) and cell-matrix adhesion (GO:0007160). The extracellular matrix (ECM) is a substrate for cell adhesion, growth, and differentiation, which plays an important role in force transmission for muscle contraction, maintenance, and repair, emphasizing its importance for REA. In relation to BFT, ECM plays an important role in adipogenesis.

Conclusion: The approaches used in this study allowed us to identify co-expressed networks correlated with important economic traits, collaborating to better understand the biological processes involved in muscle development and fat deposition in beef cattle.

Keywords: backfat thickness, ribeye area, WGCNA

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137: IDENTIFICATION OF GENOMIC REGIONS RELATED TO PH IN NELLORE BEEF CATTLE

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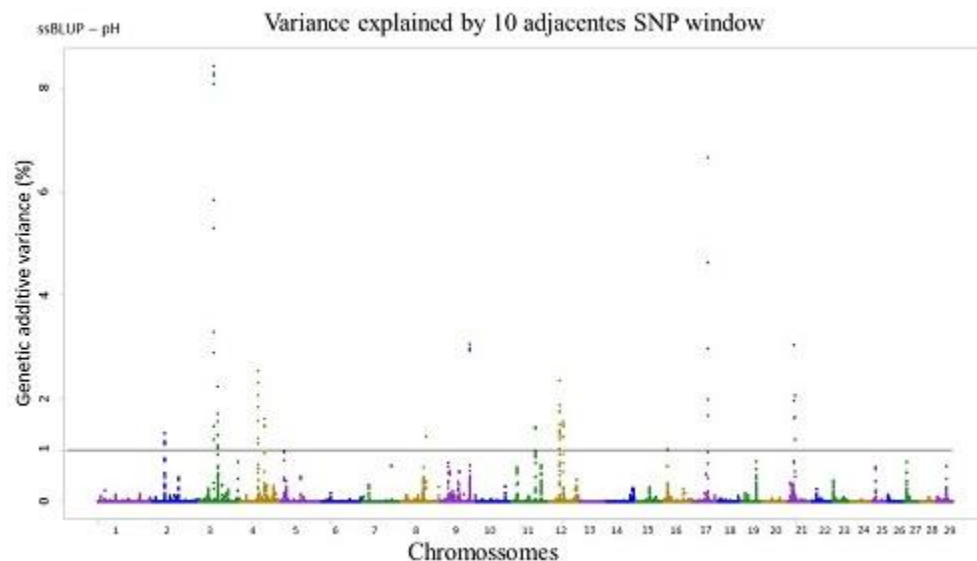
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Objectives: The aim of this study was to identify genomic regions that potentially have association with pH in Nellore cattle.

Materials and Methods: Post mortem muscle metabolism (pH) was measured on samples of Longissimus thoracis muscle collected 24 hours post mortem using a portable pHmeter (Hanna HI9963) from 1,208 feedlot Nellore steers slaughtered at two years of age. Genotypic data of all animals was used for genome wide association studies through the ssGWAS method. Those animals were genotyped with the Illumina Bovine beadchip HD® GGPI (74K). Based on another Nellore population genotyped for Illumina beadchip BovineHD® (777K), genotypes were imputed using the FImpute software. Analyses were performed using a pedigree composed by 6,276 animals. Single step analyses were performed using the Blupf90 family of programs considering windows of 10 markers to estimate the SNP effects. This procedure enables the identification of regions associated with pH along the chromosomes. After quality control (MAF <0.05%, call rate <90%), 463,995 SNPs in autosomal chromosomes were used in the association analyses.

Results: The current investigation revealed 15 regions located in 10 different chromosomes (2, 3, 4, 8, 9, 11, 12, 16, 17 and 21), which explained more than 1% of the additive variance (Figure 1). Gene identification was carried out using the BioMart do Ensembl Genome Browser tool (www.ensembl.org). The following genes were identified in six distinct regions: *WD repeat*, *sterile alpha motif and U-box domain containing 1*; *palmdelphin*; *insulin like growth factor binding protein 1*; *Bos taurus charged multivesicular body protein 7*; *tumor necrosis factor receptor superfamily, member 10d*, *decoy with truncated death domain*; *rho-related BTB domain-containing protein 2*; *spectrin repeat containing nuclear envelope protein 1* e *ubiquitin specific peptidase like 1*.

Image:



Conclusion: The ssGWAS method, using high density panel, allowed the identification of regions and genes related to pH in Nellore beef cattle. Further investigation combining information of the reported genes and its biological pathways is required to better evaluate their importance for meat quality traits.

Keywords: Bos indicus, meat quality, pH, ssGWAS

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138: INFLUENCE OF HEAT SHOCK PROTEIN ACTIVITY ON THE BEEF TENDERNESS DURING AGING

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Objectives: The aim of this study was to evaluate aging effects on tenderness and Heat Shock Proteins activity of beef muscle.

Materials and Methods: Were evaluated 303 F1 immunocastrated steers cross cattle (Nellore x South African Simmental), aged 18.0 ± 2.0 months and live weights at slaughter of 500 kg. After 24 hours post mortem, two 2.5 cm steaks were collected at the 12th and 13th ribs of the Longissimus muscle. The steaks were individually identified, vacuum packaged and aged for 1 and 14 days. Also, in each aging time, one piece of meat was cut and immediately frozen in liquid nitrogen for further Heat Shock Protein (HSP) analysis. The meat samples were analyzed for Warner Bratzler Shear Force (WBSF) according AMSA (1995) and the HSP quantification were determined by Bovine HSP 27 and HSP 70 ELISA kits (Mybiosource). The total of protein content was calculated by Bradford method. Beef samples were classified into two groups according WBSF values at 14 aging days: Tender (<4.0 kg) and Tough (>5.2 kg) and after then, were selected 20 samples from each group for HSP quantifications. Data were analyzed using GLM procedures of SAS, LS MEANS statement and the TUKEY adjustment were used for mean separation with an alpha level of 0.05 (Version 9.2, Cary, N.C., 2002 – 2008).

Results: As expected, WBSF values (n=300) at 14 aging days from Tender group were smaller (4.2 kg) than Tough group (5.3 kg; P<0.05). The HSP 27 values decreased from 1 to 14 aging days inside the Tender and Tough groups, but no differences were detected between groups at the same aging day. With respect of the HSP 70 values, were observed differences only inside the Tender group and instead of HSP 27, the values increased from 1 to 14 aging days (Table 1).

Table 1. Heat Shock 27 and 70 protein expression (pg/ mg protein) by aging time.

Image:

		Aging Time			
		1 day		14 days	
		Mean	SEM	Mean	SEM
HSP 27	Tender	185.54 ^A	9.53	156.76 ^B	11.46
	Tough	198.90 ^A	9.53	149.76 ^B	11.46
HSP 70	Tender	21.40 ^A	1.87	31.55 ^B	1.75
	Tough	25.72 ^A	1.87	27.84 ^A	1.75

Conclusion: In conclusion, there are no evidence of relationship between HSP 27 activity and meat tenderness but with respect to HSP 70 activity, more studies should be conducted to elucidate this question.

Keywords: HSP27, HSP70

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139: AGING, ANTIOXIDANT-ENHANCEMENT, AND MODIFIED ATMOSPHERIC PACKAGING IMPROVES APPEARANCE OF DARK-CUTTING BEEF

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Objectives: Color is the most crucial component of a consumer's decision when purchasing beef. Due to their dark appearance, dark-cutters will be discounted at the packing facility. Improving the appearance can increase the value of dark-cutting beef resulting in a greater profit for producers and retailers. The objective of this study was to evaluate the effects of wet-aging, antioxidant-enhancement, and modified atmospheric packaging on color of dark-cutting beef during simulated retail display.

Materials and Methods: No-roll dark cutting (pH > 6.0) strip loins (n = 12) and 10 USDA choice (pH range 5.45 – 5.55) strip loins (IMPS #180) were randomly selected from a commercial purveyor within 3 d post-harvest. Dark cutting loins were sectioned into two equal sections and assigned to one of three aging periods 7, 14, and 21 d, then cut into three equal sections and assigned to 1 of 3 treatments, control, 0.1% rosemary, and 0.2% rosemary. Choice loins were sectioned into three equal sections and randomly assigned to respective aging periods. Following aging, loins assigned to rosemary enhancement treatments were enhanced to 110% their green weight with a solution consisting of deionized water and 1.1% or 2.2% oleoresin rosemary (Kalsec Herbalox®). Following aging and enhancement, sections were sliced into 1.9-cm steaks and assigned to one of three packaging treatments; high-oxygen modified atmospheric packaging (HiOx-MAP; 80% O₂ & 20% CO₂), carbon-monoxide (CO-MAP; 0.4% CO, 69.6% N, & 30% CO₂) and polyvinyl chloride overwrap (PVC; 20% O₂). Steaks were on display under continuous fluorescent lighting for 5 d. The surface color was measured utilizing a HunterLab Miniscan XE Plus spectrophotometer each day of display. Lipid oxidation was determined on 0 and 5 d of display utilizing the thiobarbituric acid reactive substances (TBARS) assay. Data were analyzed using the Mixed Procedure of SAS and values were considered significant at P < 0.05. The experiment was replicated eight times (n = 8).

Results: The combination of aging, modified atmospheric packaging, and antioxidant-enhancement improved (P < 0.05) redness (a* values) and lightness (L* values) of dark-cutting beef compared with control dark-cutting beef. HiOx-MAP packaging was the most effective (P < 0.05) in improving surface color compared with CO-MAP and PVC packaging.

Conclusion: The results indicate that the combination of post-harvest technologies such as aging, antioxidant enhancement and packaging has potential to improve surface color and value of dark-cutting beef.

Keywords: aging, antioxidant, dark-cutter, enhancement , modified atmospheric packaging

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140: SMALL HEAT SHOCK PROTEIN EXPRESSION DIFFERS DURING AGING IN STEAKS FROM THE LONGISSIMUS LUMBORUM OF CATTLE THAT RECEIVED DIFFERENT GROWTH PROMOTANTS

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Objectives: Administration of growth-promoting technologies (GP), such as implants and beta-agonists, to feedlot animals is known to positively impact feedlot performance, however these technologies can have a negative impact on meat tenderness. Rate and extent of post-mortem proteolysis determines meat tenderness. Heat shock proteins (HSP) may play a role in this process through the protective effects these molecules exhibit over myofibrillar proteins during the degradation process. The objective of this study was to determine the effect of different growth-promoting technologies (GP) on HSP abundance in Longissimus lumborum (LL) steaks during aging.

Materials and Methods: Steaks were collected from the LL of feedlot heifers that received one of three different treatments (n = 11 per treatment) during the feedlot phase: no anabolic implant or beta-agonist (CON), anabolic implant but no beta-agonist (IMP), or an anabolic implant and a beta-agonist (COMBO). Heifers designated to receive an implant were administered Component TE-200 and the COMBO group received 8.3 mg/kg of zilpaterol hydrochloride for the final 21 d of feeding followed by a 3-d withdrawal period. At approximately 72 h postmortem, 5.08-cm thick roasts were cut and aged for 3, 14 and 35 d. At the end of the assigned aging period, a 2.54 cm steak was cut, frozen in liquid nitrogen and stored for subsequent analysis. Protein was extracted using a modified RIPA buffer. Western blot analyses were completed to measure abundance of HSP70 (HSPA) and HSP β 1 using bovine specific primary antibodies from Cell Signaling Technologies®. Statistical analyses were completed using SAS version 9.4. The statistical model analyzed repeated measures with GP treatment as the fixed effect, individual steaks as a random effect, day of aging as the repeated measure, and individual steak as the experimental unit. Differences in HSP abundance due to GP treatment were considered significant at $P \leq 0.05$.

Results: Abundance of HSP β 1 was different ($P < 0.001$) between steaks from the three different treatment groups, however there was no significant effect ($P > 0.50$) found in the interaction between treatment and day of aging. At both 3 d and 14 d of aging, there was increased ($P < 0.05$) abundance of HSP β 1 in IMP and COMBO when compared to CON steaks. At 35 d of aging, COMBO steaks had increased ($P < 0.05$) abundance of HSP β 1 when compared to CON steaks, however, abundance of HSP β 1 did not differ ($P \geq 0.26$) between IMP steaks and the CON and COMBO treatments. There was no effect ($P = 0.81$) of treatment or the interaction between treatment and day on HSPA abundance during the aging period.

Conclusion: Provision of different GP to feedlot cattle alters abundance of HSP β 1 in LL steaks during the aging period when compared to LL steaks from animals that did not receive any GP. These data may provide new insights into the mechanism through which meat tenderness is altered when these GP are administered to feedlot cattle.

Keywords: aging, growth promotants, heat shock proteins, HSP β 1, meat tenderness

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141: IMPACTS OF BOVINE MATERNAL NUTRITION ON MIRNA EXPRESSION IN SKELETAL MUSCLE OF THE PROGENY DURING GROWTH

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Objectives: Decreased gestational nutrition has been shown to alter deposition of adipose tissue in the offspring within several different livestock species. Currently, little is known pertaining to the cellular mechanism(s) that are responsible for these observed changes in adipose deposition and skeletal muscle growth. This study investigated whether progeny from cows with restricted nutrition during the second trimester had different expression of microRNA known to be involved in either skeletal muscle or adipose deposition in the skeletal muscle during growth when compared to progeny from non-restricted cows.

Materials and Methods: Cows were all bred by the same Angus sire, stratified by body weight ($P=0.80$) and body condition score (BCS) ($P = 0.72$) and allocated to one of two different treatment groups: maintenance ($n = 16$) or restricted ($n = 18$). Restricted cows (REST) were provided with lower forage biomass (1662 kg/ha, dry matter) in comparison with maintenance (MAINT) (2309 kg/ha, DM). Restricted cows had a mean BCS 1.55 lower ($P = 0.001$) than MAINT at the end of the period and a weight difference of 188 Kg ($P = 0.024$). After the second trimester all cows and their subsequent calves were treated similarly. At the beginning of the feedlot stage skeletal muscle biopsies were collected from the offspring from the *biceps femoris* (BF) and immediately snap frozen in liquid nitrogen. Additionally, samples were collected from the offspring from the *longissimus lumborum* (LD) within 20 min of harvest and snap frozen in liquid nitrogen. Expression of miR-1, -133a/b, -206, -181d, -27b, -424, -486, -214, and let-7g was analyzed using quantitative real-time PCR methods.

Results: Offspring from REST cows expressed more ($P < 0.05$) MiR-133a, -133b, -206, -214, -424 and -486 in the BF at the beginning of the feedlot phase when compared to offspring from MAINT cows. There was no difference ($P \geq 0.12$) in expression of MiR-1, -27b, or -181d in the BF between the two treatment groups at this phase of growth. Furthermore, at harvest, offspring from REST cows expressed more ($P < 0.05$) MiR-133a and -486 in the LD than offspring from MAINT cows. Offspring from REST cows also have a tendency ($P = 0.09$) for increased expression of MiR-133b in the LD when compared to offspring from MAINT cows. No differences ($P \geq 0.44$) in expression of MiR-1, -27b, -181d, -206, -214, -424, or -486 were detected in the LD immediately following harvest between offspring from either the REST or MAINT cows.

Conclusion: These data provide novel insight into alterations in microRNA expression in the skeletal muscle during growth from offspring born from cows with restricted nutrient intake in the second trimester. Offspring born from REST cows expressed more MiRNA involved in both adipose and skeletal muscle growth which is likely involved in the cellular mechanism(s) that ultimately determine meat quality through their effects on skeletal muscle growth and adipose deposition. However, further research needs to be completed to determine the exact role that these microRNA have in skeletal muscle growth and adipose deposition.

Keywords: fetal programming, growth, MiRNA, skeletal muscle

Muscle and Lipid Biology and Biochemistry

142: B-ADRENERGIC AGONIST MEDIATED CHANGES IN MUSCLE ENERGY METABOLISM IN AMPK γ 3R200Q PIGS

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Objectives: The β -adrenergic agonist Ractopamine (RAC) repartitions nutrients from adipose to skeletal muscle and enhances muscle accretion in a fast-fiber type specific manner. In contrast, muscles from pigs with a mutation in the key energy sensor, AMP-activated protein kinase (AMPK), exhibit a slower, more oxidative phenotype and elevated glycogen. Our objective was to utilize RAC and AMPK-mutated pigs to investigate the cellular signaling pathways regulating energy metabolism.

Materials and Methods: At approximately 90 kg, wild type and AMPK γ 3^{R200Q} barrows (n=29) were assigned to control diet or diet supplemented with RAC (9 ppm; Elanco Animal Health) for 28 d. Pigs were harvested and muscle was collected from the longissimus lumborum (LL) immediately after exsanguination (t=0 min) and deep (red) and superficial (white) portions of the semitendinosus (RST and WST respectively) were collected at 15 min. Glycogen content and parameters of glycogen metabolism were analyzed, and muscle metabolic phenotype was assessed by measuring glycolytic and oxidative enzyme activities.

Results: Regardless of RAC or muscle, AMPK γ 3^{R200Q} increased glycogen ($P<0.001$) and glycolytic potential ($P<0.001$) compared to wild type. In the LL, RAC decreased glycolytic potential in AMPK but not wild type (genotype x diet, $P<0.05$). Dietary RAC decreased glucose in RST and LL ($P<0.05$) and WST ($P=0.08$). The glycolytic capacity, indicated by lactate dehydrogenase activity, was differentially influenced by RAC and genotype in WST (genotype x diet, $P<0.02$), whereas in the LL, lactate dehydrogenase was increased by RAC ($P = 0.05$). In contrast, oxidative capacity, assessed by citrate synthase activity, was increased ($P<0.001$) in AMPK regardless of RAC in both LL and STW.

Conclusion: While AMPK γ 3^{R200Q} is associated with altered glycogen storage and a more oxidative metabolism, RAC administration affects muscle metabolic characteristics and alters metabolites towards glycolytic usage in a muscle specific manner. These results indicate that AMPK and RAC influence glycogen storage and energy metabolism, which may ultimately impact capacity for muscle growth.

Keywords: AMP-activated protein kinase, B-adrenergic agonist, muscle metabolism

Muscle and Lipid Biology and Biochemistry

143: PROTEOLYTIC EFFECTS OF MARINADES CONTAINING ACTINIDIN

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Objectives: Kiwi fruit contains the cysteine protease actinidin, which belongs to the same class of enzymes as papain and bromelain. Several studies have shown that meat becomes more tender when injected with these enzymes. The objective of this study was to evaluate the proteolytic effects of actinidin in bovine *semitendinosus* muscle at high (6.4) and low (5.5) pH conditions, by monitoring degradation of desmin and myosin light chain.

Materials and Methods: Kiwi fruit powder (OT-1005X), which contains the proteolytic enzyme actinidin, was obtained as a gift from the producer (Ingredient Resources Pty Ltd, Australia). Four different marinades (A-D) were prepared. Na₅P₃O₁₀ was used to obtain the high pH-marinades. Marinade **A**: 0.5% kiwi powder, 3% phosphate and 3% NaCl. Marinade **B**: 0.5% kiwi powder and 3% NaCl. Marinade **C**: 3 % phosphate and 3% NaCl. Marinade **D**: 3% NaCl. From a commercial abattoir *M. Semitendinosus* from 6 young bulls (Norwegian Red) were purchased. Four days post mortem the muscles were cut into 5 slices (approximately 3.5 cm thick), and pH was measured. One slice from each muscle was further cut into small pieces (approximately 2 mm wide). The other slices were injected to 110% weight with the A-D marinades and kept at 4°C in sealed plastic bags for 14 days. Then Warner-Bratzer (WB) shear force was measured on these muscle slices after cooking in water bath at 70°C for 50 minutes. The finely cut muscle samples, from the 5th slice, were mixed with marinades (A-D) and stored in tubes for either 3 hours, 3 days or 14 days. At the given times excess liquid was removed and muscle samples were frozen. Then the meat samples were homogenized in Tris-EDTA buffer. Degradation of desmin and myosin light chain were measured by Western blotting of SDS-PAGE gels. Protein bands were quantified with the Image Quant software (GE Healthcare). Analysis of variance (ANOVA) was performed with the software Minitab (Version 17.2.1).

Results: There was no difference ($p>0.05$) in the pH values, which was in the range 5.55 to 5.62 (s.d 0.04), of the six *semitendinosus* muscles used in this study. Marinade A and C had pH-values of 6.38 and 6.45 respectively after addition of muscle, which were higher ($p<0.001$) than the corresponding values for marinade B and D which were 5.49 and 5.52. The muscle slices injected with marinade A had 34.0 (s.d 8.8) N cm⁻² as average WB shear force value, which was lower ($p<0.05$) than slices injected with marinade D (49.0 N cm⁻², s.d 8.8). Average WB values for the samples injected with marinade B and C were 44.7 (s.d 10.4) and 40.3 (s.d 5.2) N cm⁻² respectively, and these were neither different from marinade A or D. Both desmin and myosin light chain were increasingly degraded with time ($p<0.001$), but no difference was found between the marinades A-D at each of the three time periods.

Conclusion: This study has shown that actinidin has a tenderizing effect on bovine *semitendinosus* muscles. The proteolytic activity seems to be higher when pH is around 6.4 than 5.5. Since no differences were seen in degradation rate of desmin and myosin light chain between the 4 marinades, the proteolytic activity of actinidin is limited against these proteins.

Keywords: actinidin, proteolysis, Warner-Bratzler shear force

Muscle and Lipid Biology and Biochemistry

144: MITOCHONDRIAL LIPID COMPOSITION AND ENZYME ACTIVITY OF POST MORTEM BEEF LONGISSIMUS MUSCLE FROM ANGUS STEERS FED ENDOPHYTE-INFECTED TALL FESCUE SEEDS.

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Objectives: The objective of the study was to determine the effects of feeding endophyte-infected tall fescue seeds on mitochondrial fatty acid (FA) composition and phospholipid (PL) fractions and activity of superoxide dismutase (SOD) and metmyoglobin reductase (MRA) in beef *longissimus* muscle from Angus steers.

Materials and Methods: Twelve Angus steers were blocked by initial BW and randomly assigned to be fed with either KY32 (E- or control) or KY31 (E+ or treatment; approximately 20 µg of ergovaline per kg of BW) within a block. Steers were fed individually using Calan® gates in the first 70-d trial in the summer of 2015, followed by a 149-d withdrawal period and the second 64-d trial in the winter of 2016. After the second trial, steers were implanted with a dose of Ralgro®, finished for 66 d on summer perennial pastures, and slaughtered at approximately 500 kg of BW. Immediately after carcass decontamination, *longissimus thoracis* muscle was collected at the 12th rib on the left side of the carcasses, cubed, snap-frozen in liquid nitrogen, wrapped in aluminum foil, vacuum-packaged, and stored at -80°C for FA, PL, and SOD analyses. Strip loins were collected at 72 h post-mortem, aged for 14 d, trimmed to 0.3-cm backfat thickness, cut into 2.54-cm steaks, placed on black Styrofoam® trays, overwrapped with PVC film (O₂ permeability of 1.21 mL/cm²/d and water vapor permeability of 0.022 g/cm²/d; LINPAC Packaging-Filmco Inc., Aurora, OH), and placed under simulated retail display conditions (2 to 4°C, 900-lux fluorescent intensity, and 80% relative humidity) for 0, 1, 3, 5, and 7 d. One steak per animal per time point was collected for MRA analysis. Mitochondria were separated by ultracentrifugation and their lipids were extracted in 1:2 chloroform:methanol (v/v) and converted to fatty acid methyl esters to be analyzed by gas chromatography (Hewlett-Packard 6890 FID GC System; Agilent Technologies, Santa Clara, CA). Phospholipid classes were determined by thin-layer chromatography. Activity of SOD was determined by a colorimetric assay kit applicable for muscle (ab65354; Abcam, Cambridge, MA). Metmyoglobin reducing activity (µM of MMb reduced/min/g of muscle) was determined by reacting extracted muscle reductases with equine skeletal metmyoglobin and measuring deoxymyoglobin at 580 nm (Spectra max plus 384; Molecular Devices, Sunnyvale, CA). One steer with a large abscess, yielding pH of 6.35 and dark cuts, was excluded. Statistical analysis was performed by the GLIMMIX procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) at 0.05 level of significance.

Results: Feeding endophyte-infected tall fescue seeds did not affect mitochondrial FA composition, PL fractions, and SOD activity ($P \geq 0.14$). Metmyoglobin reducing activity of E+ steaks was 6.01 ± 0.37 µM/min/g, similar to that of E- steaks (6.92 ± 0.41 µM/min/g; $P = 0.117$). As expected, MRA was correlated with length of retail display ($r = -0.74$; $P < 0.001$) and decreased from 9.54 ± 0.49 µM/min/g on d 0 to 2.29 ± 0.93 µM/min/g on d 7 ($P < 0.001$).

Conclusion: Endophyte-infected tall fescue may not affect the integrity of mitochondria and MRA. A decrease in color stability was well correlated with decreased activity of metmyoglobin reductase.

Keywords: tall fescue, beef, metmyoglobin reducing activity, mitochondria

Muscle and Lipid Biology and Biochemistry

145: EFFECT OF LOCATION AND POSTMORTEM AGING ON TENDERNESS AND SARCOMERE LENGTH OF BEEF LONGISSIMUS LUMBORUM AND SEMITENDINOSUS STEAKS

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Objectives: The purpose of this study was to determine the effect of steak location and postmortem aging on cooked meat tenderness and sarcomere length of steaks from the *Semitendinosus* (ST) and *Longissimus lumborum* (LL).

Materials and Methods: Forty crossbred steers were processed at a commercial facility, and from one side of each carcass, the ST and LL subprimal were collected. Each ST was divided into 5 locations (LOC) with LOC 1 being the most proximal and LOC 5 the most distal. Similarly, each LL was divided into 3 LOC with LOC 1 starting within the middle of the subprimal and LOC 3 being the most posterior. Within each subprimal location a 2.54-cm thick steak was fabricated for Warner-Bratzler shear force (WBSF) and sarcomere length analyses. Steaks from each LOC within the ST were randomly assigned to 7, 14, 28, 56, or 112 d of aging (DOA) and steaks from each LL LOC were randomly assigned to 7, 28, or 112 d of aging. After the appropriate aging period, WBSF and sarcomere steaks were cooked to 71°C and chilled overnight. Six cores were removed parallel to muscle fibers and were sheared perpendicular to the muscle fibers. Sheared cores were frozen and powdered for sarcomere length. The Z- and M-Lines were marked under a laser beam, and the equation established by Cross et al., (1981) was utilized to quantify sarcomere length. Data were analyzed as a randomized complete block design with repeated measures.

Results: There were no LOC × Day of aging interactions for ST or LL steaks for WBSF or sarcomere length ($P > 0.25$). Steaks from the ST had reduced WBSF values in LOC 4 compared to other LOC ($P < 0.05$). The ST steaks for LOC 1 and 2 had the greatest WBSF values compared to the more distal LOC ($P < 0.05$). Sarcomere length was shorter for ST steaks from LOC 1 and 2, compared to LOC 4 and 5 ($P < 0.05$). Longer sarcomere lengths were found in the more distal LOC for ST steaks than in the proximal LOC ($P < 0.05$). The LL steaks were not different in WBSF values across LOC ($P > 0.05$). Additionally, sarcomere length of LL steaks was not different across LOC ($P > 0.05$). As day of aging increased, WBSF values decreased in ST steaks ($P < 0.05$). However, no differences ($P > 0.05$) were detected for sarcomere length in ST steaks as day of aging increased. In LL steaks, WBSF values decreased and sarcomere length increased ($P < 0.05$) as day of aging increased.

Conclusion: *Semitendinosus* steaks from the center portion of the subprimal were instrumentally more tender than proximal steaks, but LOC had no effect on instrumental tenderness for LL steaks. Increased day of aging resulted in more tender ST and LL steaks. To provide the highest eating satisfaction to consumers on a budget, extended aging and steak location should become most valued when selecting ST steaks. While loin steaks can be more consistent in tenderness, consumers can value quality over steak location.

Keywords: aging, beef, location, shear force, tenderness

Muscle and Lipid Biology and Biochemistry

146: FATTY ACID COMPOSITION OF MEAT RETAIL DISPLAY FROM NELLORE STEERS FED DIFFERENT OIL SOURCES

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Objectives: The aim was to evaluate the fatty acids (FA) composition of meat samples exposed to retail display conditions for three days from Nellore steers fed different oil sources during the feedlot finishing.

Materials and Methods: Ninety-six Nellore (*Bos indicus*) steers were fed for 81 days with diets containing different oil sources: soybean (SOY – 6.6% ether extract - EE), sunflower (SUN – 6.9% EE), linseed (LIN – 6.8% EE) and a diet control (CON - without addition of oil - 3.5% EE). Diets were composed of 21% corn silage and 79% concentrate (dry corn grain, soybean meal, citrus pulp, urea, mineral nucleus and calcitic limestone) and inclusion of oils (3.5%) was made by partial substitution of corn grain. After 81 days of feeding, animals were slaughtered (507.5 ± 17.3 kg LW and 5.2 mm of backfat thickness) and samples of *longissimus* muscle (2.5 cm thick) were collected at 12th rib level after 48 hours *postmortem*. Steaks were placed on Polyfoam trays, overwrapped with an oxygen-permeable polyvinylchloride film and stored for three days under simulated retail display conditions of illumination (Halogen light; 2000 lx) and temperature (0 - 2 °C). After this period, steaks were frozen and analyzed for FA composition using the methods by Folch et al. (1957) and Kramer et al. (1997), and quantified using a gas chromatography. The experiment was set up as a completely randomized block (initial body weight) design and analyzed using the mixed model, considering diets as fixed effects.

Results: No effect ($P > 0.05$) of oil addition in diet was observed for most FA percentage (average among all treatments): c9 t11 CLA (0.42% ± 0.04) and total concentrations of monounsaturated (45.17% ± 0.86), saturated (SFA) (41.13% ± 0.75), polyunsaturated (PUFA) (10.06% ± 0.85), n-3 (1.50% ± 0.20), n-6 (8.3% ± 0.70). The c6 18:1 concentration was higher ($P = 0.04$) in meat from steers fed LIN (0.36% ± 0.03) and SOY (0.37% ± 0.03), in comparison to CON (0.19% ± 0.04). This c6 18:1 increased concentration is seen as beneficial to human health because of cis positional configuration. Linolenic acid was in higher concentration ($P = 0.01$) in animals fed LIN (0.96% ± 0.05) compared to other treatments (0.46% ± 0.05), which would be expected due to the high concentration of 18:3 n-3 in linseed oil. This is a desirable result, because the goals of feeding LIN were to increase n-3 FA in the meat because of their benefits to human health. In consequence, meat from LIN fed animals had a high n-6:n-3 ratio (4.15 ± 0.55) which is close to the recommended ratio (4.0) by the World Health Organization – WHO (2003). The meat of animals fed SUN and SOY showed n-6:n-3 ratio of 8.75 and 7.66, respectively, which are higher than recommended by the WHO. Despite no differences among treatments for n-6 FA, the high n-6:n-3 ratio observed for SUN and SOY occurred probably due to the high amount of 18:2 n-6 in these oils. The PUFA:SFA (0.25 ± 0.02) ratio was not affected by diets ($P > 0.05$) and was below the recommended ratio which is greater than or equal to 0.4. The index of enzymes activity $\Delta 9$ desaturase C16 (11.05% ± 0.36), $\Delta 9$ desaturase C18 (72.07% ± 1.18) and elongase (65.48% ± 0.50) were not influenced by diets ($P > 0.05$).

Conclusion: The LIN diet provided meat with better FA composition considering the higher concentration of linolenic acid and adequate relation of n-6:n-3, which is positive for human health.

Keywords: beef, linolenic acid, longissimus muscle, vegetable oil

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147: EXCESS GLYCOGEN DOES NOT RESOLVE HIGH ULTIMATE PH OF BEEF, LAMB, AND CHICKEN OXIDATIVE MUSCLE

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Objectives: Glycogen is the main energy source during the conversion of muscle to meat. Lower glycogen levels of oxidative muscle antemortem were thought to contribute to a higher ultimate pH. However, excess glycogen did not resolve the high ultimate pH of porcine *masseter* (oxidative) muscle. To understand this phenomenon further in other species, we hypothesized that excess glycogen may not resolve the high ultimate pH of oxidative muscles in beef, lamb, and chicken.

Materials and Methods: Six market-weight beef cattle, lambs, and chickens were harvested at The Ohio State Meat Center under USDA-FSIS supervision. *Cutaneous trunci* (glycolytic) and *masseter* (oxidative) muscle samples from the ruminants, and *pectoralis major* (glycolytic) and *sartorius* (oxidative) muscle samples from the chickens were collected immediately after exsanguination. The samples were snap frozen in liquid nitrogen and stored at -80°C until further analysis. Muscle samples were powdered in liquid nitrogen and homogenized into an anaerobic glycolysis buffer containing 10 mM Na₂HPO₄ (pH 7.4), 5 mM MgCl₂, 60 mM KCl, 5 mM ATP, 0.5 mM ADP, 0.5 mM NAD⁺, 30 mM glycogen, 25 mM carnosine, 30 mM creatine, and 10 mM sodium acetate at 100 mg muscle/mL. Reaction vessels were placed in a dry heating block at 25°C and aliquots were removed at 0, 30, 60, 120, 240, and 1440 min for pH, glycogen, glucose 6-phosphate, glucose, and lactate analysis. Data were analyzed with a mixed model in JMP. Individual animals were recognized as an experimental unit and time-course data were analyzed with a split-plot design. The least squares means were evaluated with a Student's t-test and considered significant at $P \leq 0.05$.

Results: Glycogen content between muscle homogenates was similar at 0 min in all the species and decreased significantly ($P < 0.001$) with time. However, both glycolytic and oxidative muscle homogenates contained residual glycogen at 1440 min which indicated that glycogen was not completely depleted in all species tested. The muscle homogenate pH decreased ($P < 0.001$) with time in all species. However, the ultimate pH at 1440 min of the oxidative muscle homogenates was significantly ($P \leq 0.023$) greater than the glycolytic muscle homogenates in all species tested. Lactate content increased ($P < 0.001$) with time in all muscles, but the oxidative muscle homogenates contained decreased ($P \leq 0.0231$) lactate levels at 1440 min in all species tested. Glucose 6-phosphate content increased significantly ($P < 0.001$) from 0 to 30 min in both muscles of all species tested, followed by relatively consistent levels until 240 min. There was again a significant ($P \leq 0.023$) increase in glucose 6-phosphate levels from 240 to 1440 min in all the muscles, however the levels were significantly ($P \leq 0.005$) lower in oxidative muscles as compared to glycolytic muscles in all the species tested. These results are consistent with the previous findings in pigs.

Conclusion: Combined these data indicate that glycolysis and pH decline terminate prematurely in the presence of excess glycogen in postmortem oxidative muscles across livestock species.

Keywords: beef, chicken, glycogen, pork, ultimate pH

Muscle and Lipid Biology and Biochemistry

148: METHOD FOR ELECTROPHORETIC SEPARATION OF BOVINE MYOSIN HEAVY CHAIN ISOFORMS

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Objectives: Myosin heavy chain (MyHC) isoform composition is a primary determinant of contractile speed of muscle fibers. Current methods for assessing bovine MyHC isoforms involve time-consuming histochemical evaluation by immunofluorescence or ATPase activity. Alternatively, electrophoretic separation of MyHC isoforms is more rapid, and this technique has been utilized in mice, pigs, and other species. Therefore, our objective was to establish a reliable procedure for separating bovine MyHC isoforms (I, IIa, and IIx) using sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), followed by validation with Western blotting and histochemical analyses.

Materials and Methods: Muscle samples were collected from beef carcasses within 1.5 h after exsanguination; samples were processed for SDS-PAGE and immunohistochemical determination of fiber type and size. Muscles were chosen to represent a variety of fiber type compositions, including masseter, superficial pectoral, longissimus lumborum, and cutaneous trunci. For SDS-PAGE, proteins were extracted using a sodium phosphate SDS buffer. To determine appropriate conditions for MyHC isoform separation, the following parameters were evaluated: percent acrylamide (7-9% for separating; 4-5% for stacking) and acrylamide to bis-acrylamide ratios (50:1 and 37.5:1), glycerol concentrations (30-45%), and electrophoresis running buffers. After SDS-PAGE, proteins were stained with Coomassie to validate all three isoforms were separated. Once conditions were established, MyHC composition was calculated using band intensity for each isoform relative to total intensity of all three types. In addition, Western blotting was used to confirm identity of MyHC isoforms. Primary antibodies (Developmental Studies Hybridoma Bank; Iowa City, IA) were of unique isotypes to detect a combination of MHC using 2 color detection. A primary antibody for all MHC types (MF 20; IgG2b) was used in conjunction with BF-32 (MHC I and IIa; IgM), A4.840 (MHC I; IgM), SC-71 (MHC IIa; IgG1), or 6H1 (MHC IIx; IgM). Primary antibody BA-F8 (MHC I; IgG2b) alone was also used. In conjunction, relative area of muscle fiber types was calculated using immunohistochemical determination of MyHC composition and cross sectional area. Muscle cross sections were incubated with primary antibodies (BA-F8 and BF-32), followed by AlexaFluor conjugated secondary antibodies; AlexaFluor 488 conjugate to wheat germ agglutinin was used to visualize muscle cell membranes. Fiber CSA and area were determined using ImageJ software.

Results: All three bovine MyHC isoforms were separated using a discontinuous gel system. The separating gel consisted of 37% glycerol, 8% acrylamide-bis (50:1), 0.2 M Tris (pH 8.8), 0.1 M glycine, and 0.4% SDS, and the separating gel was composed of 37% glycerol, 4% acrylamide-bis (50:1), 70 mM Tris, 4 mM EDTA, and 0.4% SDS. Ammonium persulfate and TEMED were used to initiate polymerization of separating and stacking gels. Electrophoresis was performed at 80V for approximately 40 hours at 4 C. Identity of isoforms was confirmed with Western blotting, and percent MyHC composition evaluated by SDS-PAGE was consistent with relative area determined by immunohistochemistry ($P < 0.05$).

Conclusion: Modification to SDS-PAGE parameters results in clear and consistent separation of bovine MyHC isoforms, thereby providing a more rapid means for determining MyHC composition compared to histochemical methods.

Keywords: muscle fiber type, myosin heavy chain