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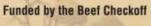
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#### 1

#### ALTERNATIVE MERCHANDISING STRATEGY OF THE BEEF TOP ROUND

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#### **Objectives: Alternative Merchandising Strategy of the Beef Top Round**

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Whole top rounds can lack color uniformity causing discounts at the retail counter and profit loss due to color having a large influence on consumers' willingness to purchase. The primary objective of this study was to compare the deep portion, superficial portion, and whole beef top round steaks (semimembranosus (SM) muscle) for consumer willingness to purchase based upon visual appraisal of steak pictures.

**Materials and Methods:** USDA Choice top rounds (IMPS 168; n = 12) were purchased from a commercial food distributer and aged between 21 to 24 days from their pack date before removing the SM. Five steaks were cut from each SM proximally to distally and systematically assigned to D0, 1, 2, 3, or 4 days of retail display. Steaks were displayed in a glass-fronted retail display case at 3°C. On the assigned day of retail display, whole steaks were pictured 15 inches above the steak. This was then followed by separation of the deep/superficial portions approximately five cm from the steak's superficial edge. Individual pictures of the deep and superficial portions were then taken as described above. The survey was distributed online though Qualtrics. Pictures were randomized and each participant evaluated 18 images. After evaluating each picture, participants were asked if they would purchase the steak. If yes, they were asked how much they would be willing to pay per pound (\$2.58, \$3.58, \$4.58, \$5.58, and >\$6.58). If no, they were asked why not, with options including: amount of trim, toughness, color, and amounts of marbling. Data was analyzed using the glimmix procedure Statistical Analysis System (SAS).

**Results:** Significance was determined at (P < 0.05). A total of 265 consumers completed the survey and 3,507 purchase decisions were made. For willingness to purchase there was not an interaction (P = 0.1761) between day of retail display and steak portion. However, there was a difference (P < 0.001) between steak portions. Consumers preferred the superficial portion over the whole steak and the whole steak over the deep portion. There was also a difference (P < 0.001) between days of retail display. Interestingly, in general, consumers preferred steaks on days 1, 2, and 3 over steaks on days 0 and 4 of retail display.

**Conclusion:** In conclusion, consumers were more willing to purchase the superficial portion of the semimembranosus over the whole steak. This could allow for an alternative merchandising strategy of the top round which potentially would add value to the beef round.

Keywords: Alternative fabrication, Consumer survey, Top Round



#### 3

#### NUTRIENT PROFILE COMPARISON OF PLANT-BASED MEAT ALTERNATIVES AND GROUND PORK

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**Objectives:** Animal-based meat products are an important source of nutrients in human diets; although plant-based meat alternatives (PBMA) have been gaining popularity in recent years, and it could be an important source of nutrients for some consumers. However, a substantial knowledge gap exists about the full nutrient profile of PBMAs and ABMs. Therefore, nutrient profiles of original and current formulations of popular PBMAs such as the Beyond Meat Burger (BMB1 and BMB2), Impossible Food Burger (IFB1 and IFB2), and Morning Star's Black Bean Burger (BBB) were assessed in comparison to 80/20 ground pork (GP) for proximate, mineral, vitamin, fatty acid, and amino acid profiles. **Materials and Methods:** The BMB1, BMB2, IFB1, IFB2, BBB, and GP products were purchased at foodservice companies and supermarkets from six randomly selected cities throughout the United States. Six replicates (n = 6) of each food product (i.e., one replicated per city) were formed into 113 g patties and either left raw or cooked on pre-heated aluminum skillets to an internal temperature of 71°C. Both raw and cooked products were frozen in liquid nitrogen and immediately homogenized until a uniform powder was obtained. Homogenized samples of raw and cooked products were then analyzed for proximate, mineral, vitamin, fatty acid, and amino acid profiles according to official procedures put forth by the Association of Official Agricultural Chemists (AOAC). Simple means and standard deviations of each nutrient in each product were calculated, and statistical differences were determined at an alpha level of 0.05 using an Anova with Tukey adjusted p-values.

**Results:** Crude protein and fat content did not differ (P > 0.05) between the PBMAs and GP after cooking. Sodium, calcium, and iron content were considerably greater (P < 0.05) in all PBMAs than GP. Magnesium content in BMB2 and IFB2 were also greater (P < 0.05) than in GP, as was vitamin E content in all PBMAs, when compared to GP. IFB1 and IFB2 products were either numerically comparable to or statistically greater (P < 0.05) than GP in each B-vitamin assessed, except pantothenic acid ( $B_5$ ) for which GP was greater (P < 0.05). Total saturated and mono-unsaturated fatty acid content was numerically greater in BMB2 and IFB2 than GP. Total essential amino acid content was numerically greater in BMB2 than in GP, although anabolically important essential amino acids, such as methionine and lysine, were substantially greater (P < 0.05) in GP than in all the PBMAs evaluated.

**Conclusion:** High sodium and saturated fatty acid content in PBMAs may be a health concern for some consumers. The high concentrations of calcium, magnesium, and thiamin ( $B_1$ ) and the presence of other nutrients found in PBMAs could have implications on bioavailability. Therefore, the integration of dietary nutrients into the food matrix of PBMAs and bioavailability of nutrients should be further investigated.

Keywords: plant-based meat alternatives, animal-based meats, amino acid profile, fatty acid profile



#### 4

**CHANGES IN THE PERCEPTION OF GROUND BEEF QUALITY AS A RESULT OF PRICE PER POUND LABELING** E. S. Beyer<sup>1,\*</sup>, K. M. Harr<sup>1</sup>, S. G. Davis<sup>1</sup>, K. J. Farmer<sup>1</sup>, M. D. Chao<sup>1</sup>, J. L. Vipham<sup>1</sup>, M. R. Zumbaugh<sup>1</sup>, T. G. O'Quinn<sup>1</sup> <sup>1</sup>Animal Sciences and Industry, Kansas State University, Manhattan, United States erbeyer@ksu.edu

**Objectives:** The objective of this study was to determine the effect of perceived palatability on ground beef patties by providing consumers with differing price per pound labels.

**Materials and Methods:** Ground beef chubs (n=15) of 80% lean, 20% fat composition were procured from a beef processor from the same lot. Patties were formed 11 days after processing into 151.2g patties using a commercial patty former. Every two patties were packaged together with a four-digit identification code with one of the following price labels: Ultra-High, High, Medium, Low, Ultra-Low and no information (NONE). For all panels, samples were thawed 24 h in advance, cooked to an end-point temperature of 71°C on Cuisinart Clamshell Grill. The consumers (n=105) were asked to evaluate each sample independently with the following information provided prior to sampling: \$13.78/kg Ultra High; \$11.02/kg High; \$8.27/kg Medium; \$5.51/kg Low; \$2.75/kg Ultra-Low or no information provided. Each round, all consumers were given the same information about the price per pound for each sample. The consumers were asked to evaluate each sample for tenderness, juiciness, texture liking, flavor liking and overall liking. Additionally, the consumers were asked to list if the sample was acceptable for all traits. The consumers reported their likelihood to purchase each sample. Data were analyzed using SAS Proc GLIMMIX as a completely randomized design.

**Results:** There were no differences (P > 0.05) among any of the various price labels for tenderness, texture liking, and overall liking. Consumers were equally as likely (P > 0.05) to purchase all samples regardless of the price label. However, the consumers listed price as one of the top purchasing motivators, similar (P > 0.05) to fat content, and

appearance. Consumers found the ultra-high, medium, and ultra-low price label to be more juicy (P < 0.05) than the low price or NONE label. Also, consumers gave a higher (P < 0.05) flavor liking score to the ultra-high, high, medium, and ultra-low price labels in comparison to the NONE label. The ultra-high and medium price labels had a greater (P < 0.05) change in ratings for overall liking than the ultra-low and low price labels when compared to the NONE label.

Furthermore, almost every price label for every trait resulted in increased (P < 0.05) palatability ratings, aside from the low price label for juiciness, tenderness, and overall liking. A greater (P < 0.05) percentage of samples with the ultra-high and medium price level were rated as acceptable for juiciness in comparison to the low price and NONE label. A greater (P < 0.05) percentage of samples labeled with the ultra-high and medium price labels were considered acceptable for flavor in comparison to all other price labels. Lastly, a greater (P < 0.05) percentage of samples labeled with the ultra-high and medium price labels does be acceptable for flavor in comparison to all other price labels. Lastly, a greater (P < 0.05) percentage of samples labeled with the ultra-high, high and medium price labels were considered acceptable overall when compared to the NONE label.

**Conclusion:** Even though all samples were the same, consumer perceptions of palatability traits were influenced by price labels. While the higher price was perceived to have advantages in some quality aspects, consumers were still not more likely to purchase the higher priced sample. This indicates that even though consumers perceived the quality to be higher with a higher price label, the added quality did not justify their willingness to purchase over the lower perceived quality and priced samples.

Keywords: ground beef, labeling, palatability, price, purchasing.



#### 5

### TRAINED SENSORY EVALUATION OF PLANT-BASED GROUND BEEF ALTERNATIVES IN COMPARISON TO GROUND BEEF OF VARIOUS FAT PERCENTAGES

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**Objectives:** The objective of this study was to determine if current plant-based protein ground beef alternatives (GBA) offer similar palatability characteristics to ground beef (GB) patties of varying fat percentages.

**Materials and Methods:** Fifteen different production lots (n = 15 / fat level) of 1.36 kg GB chubs of three different fat levels (10%, 20%, and 30%) were collected from retail markets in the Manhattan, KS area. Additionally, GBA products including a soy and potato-protein based Foodservice GBA (FGBA), a pea-protein based Retail GBA (RGBA), and a Traditional soy-protein based GBA (TGBA), (n = 15 production lots / product) currently available at commercial channels were obtained from retail markets and a commercial foodservice chain. All GB and GBA treatments were hand-formed into 151-g patties and frozen at -40°C until analysis. Patties were thawed and cooked to <u>an end point temperature</u> <u>of</u> 71°C on a clamshell-style grill, cut into six wedges, and served within five minutes of cooking. Panelists (n = 120) were trained based on AMSA protocols and <u>evaluated cooked internal color, beef</u> and non-beef odor for each sample. Each sample was evaluated for juiciness, tenderness, beef flavor <u>identity</u>, beef flavor intensity, off flavor, and texture. Each characteristic was evaluated on a continuous line scale, anchors set at 0 and 100, midpoint of 50. All data were analyzed as a completely randomized design with treatment as a fixed effect.

**Results:** For all palatability traits evaluated, GB samples were different (P < 0.05) than all GBA samples. <u>All GB samples</u> were juicier, less tender, had higher beef flavor ID and intensity ratings, were firmer, and had lower off-flavor ratings than all GBA samples (P < 0.05). Of the GBA samples, TGBA was the least juicy (P < 0.05). For tenderness, all GBA samples were rated more tender (P < 0.05) than all GB samples, and for texture, all GB samples were firmer (P < 0.05) than all GBA samples. The RGBA and TGBA samples were the softest (P < 0.05). For beef flavor identity, all GB samples were rated higher (P < 0.05) than GBA samples. The GBA samples were all rated similar for beef flavor identity (P > 0.05). For beef flavor intensity, all GB samples had less (P < 0.05) off-flavor intensity than all GBA samples. There were no differences (P > 0.05) among GB samples for off-flavor intensity. Panelists described the off flavors of RGBA and FGBA as "fermented sour bean". Panelists described the off flavors of TGBA as "starchy". For color, all GBA samples rated closer to well-done appearing (P < 0.05) than all GB samples, indicating less red was visible in the internal center of the GBA samples. For beef odor, all GB samples rated substantially higher (P < 0.05) than all GBA samples. For non-characteristic beef odor, all GB samples rated much lower (P < 0.05) than all GBA samples.

**Conclusion:** These results indicate GB samples had higher ratings than GBA samples for most palatability traits evaluated. Moreover, the GBA samples did not rate similarly to GB samples for visual or odor characteristics. This clearly indicates the eating experience provided by the GBA is different than that provided by traditional GB. Thus, consumers who purchase GBA should not expect the same eating quality as they would receive with GB.

Keywords: Alternative Protein, Ground Beef, Ground Beef Alternative, Palatability, Trained Sensory Panel



#### 6

### CHANGES IN CONSUMER SENSORY EVALUATION OF GROUND BEEF WHEN INFORMATION IS PROVIDED ABOUT THE PRIMAL SOURCE

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**Objectives:** The objective of this study was to evaluate the effect of providing information about the primal source of ground beef on the palatability of 80/20 ground beef patties.

**Materials and Methods:** Chubs (n = 15) of 80% lean/20% fat ground beef were procured from a commercial purveyor and were from the same production day and lot. Utilizing a patty former, ground beef was formed into 151.2 g (approximately 13 cm diameter; 1 cm thick) patties. Chubs were randomly assigned to consumer panel sessions and paired patties from a single chub were assigned randomly to 1 of 4 different primal blend types: ground chuck, ground round, ground sirloin, and store ground plus an unlabeled sample (**NONE**) to be used for consumer sensory analysis. For consumer sensory evaluation, consumers (N = 105) evaluated each sample on 0–100-point continuous line scales for juiciness, tenderness, texture, flavor liking, and overall liking, as well as gave their purchasing intent. Additionally, each consumer was asked to rate each of the palatability traits as either acceptable or unacceptable. While all samples were the same 80/20 ground beef, consumers were provided with additional labeling information prior to their evaluation of each sample both verbally and on a screen with the NONE sample having no information provided. Finally, the change in consumer and acceptability ratings was calculated against the NONE sample. Finally, data were analyzed as a completely randomized design.

**Results:** Consumers rated ground chuck and ground sirloin more (P < 0.05) tender than ground round and NONE, but similar (P > 0.05) to store ground. Moreover, ground chuck and ground sirloin were rated higher (P < 0.05) for juiciness than NONE. For flavor liking, store ground was similar (P > 0.05) to all treatments. When evaluating texture, consumers rated ground chuck higher (P < 0.05) than ground round, store ground, and NONE, but similar (P > 0.05) for overall liking than NONE. Consumers also indicated that they would be more (P < 0.05) likely to purchase ground chuck than ground round, store ground, and NONE. When evaluating the change in consumer ratings when information was given about the sample, all treatments had an increase (P < 0.05) in ratings across all the traits evaluated. Additionally, ground chuck had a greater (P < 0.05) increase in tenderness ratings when information was provided to the consumer in comparison to ground round and store ground. There were no differences (P > 0.05) in the percentage of samples rated as acceptable for tenderness, juiciness, flavor, texture, and overall liking. However, providing information about the primal source information to consumers did not (P > 0.05) increase the percentage of samples rated as acceptable for tenderness, flavor, texture, and overall liking.

**Conclusion:** Adding a primal source to the labeling of ground beef gives consumer a sense of added quality when consuming these products over those that do not have a primal source attached to their label. In the current study, adding a primal source identification positively shifted the consumers perception of the samples provided.

Keywords: beef, consumer, ground beef, Labeling, palatability



#### IMPACT OF PLANT- AND ANIMAL-BASED PROTEINS ON SWINE ILEAL AND FECAL MICROBIOMES

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**Objectives:** The gut microbiome plays a direct role in human health and can influence health conditions varying from obesity and diabetes to cancer. Previous research has indicated that the human gut microbiome is drastically modified when fed a diet containing only animal or plant-based proteins. While most of these studies focused on traditional plant proteins (soy, legumes, etc.), none have evaluated the impact of the novel plant-based meat alternatives. Although the nutritional composition of the plant and animal proteins are comparable, a recent study suggested that the protein digestibility of animal-based products is greater when compared to their plant counterparts. Therefore, a cannulated swine model was utilized to investigate the impact of plant- and animal-based proteins on the ileal and fecal microbiome.

Materials and Methods: To best model the human digestive system, ten ileal cannulated gilts were allotted to a 10 x 6 Yourden square with 6 feeding periods of 9 days. Ileal digesta were collected for 9 h on days 8 and 9, while fecal samples were collected on days 6 and 7. Treatment diets consisting of a burger (beef 80%, lean beef 93%, ground pork, Impossible Burger, or Beyond Burger) or a combination of the burger product along with a bun (beef 80%, pork, or Impossible) were used as the only source of nitrogen in a nitrogen-free ration. In addition, a nitrogen-free diet was served as the control. The ileal and fecal samples were processed with a fecal extraction kit and sequenced on an Illumina sequencing platform. Reads were generated and bioinformatically processed with the QIIME2 pipeline, with Shannon's diversity and unweighted UniFrac phylogenetic metrics being generated with an alpha level set to 0.05. **Results:** Significant differences (*P* < 0.05) in alpha and beta microbial diversity were found between the fecal and ileal microbial communities regardless of treatment diet. Although there might be some inherent differences in protein quality, no differences (P > 0.05) in alpha diversity were identified when evaluating treatment impacts on ileal and fecal microbiomes. For beta diversity, adding a bun to the protein sources had no impact (P > 0.05) on microbial populations compared to the protein source alone. When products were grouped based on protein type (plant or animal), differences in beta diversity (P = 0.01) were identified in the ileal microbiome. Fecal samples also had similar differences (P < 0.01) between plant and animal products. However, when fecal samples were evaluated on an individual basis, all animal proteins were found to be different (P < 0.05) from plant products, except the pork burger with a bun. The pork burger with bun was similar (P > 0.05) to the Impossible burger in beta diversity.

**Conclusion:** Ileal and fecal microbiomes were different in the richness of bacteria with different species present in either group, suggesting that a fecal sample is not always representative of the whole gut microbiome. Although animal and plant-based diets were found to be similar in alpha diversity, different microorganisms were found depending on the protein source provided. Our results indicated that that digestive tract microbial communities are impacted by the type of protein consumed (plant or animal).

Keywords: 16s rRNA, Animal Protein, Fecal Microbiome, Ileal Microbiome, Plant Based Protein



#### 8

### CHANGES IN CONSUMER SENSORY EVALUATION OF 80/20 GROUND BEEF WHEN ADDITIONAL LABELING INFORMATION IS PROVIDED

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**Objectives:** The objective of this study was to evaluate the effect of providing consumers with information related to different labeling terms on the palatability ratings of ground beef patties.

**Materials and Methods:** Chubs (*n* = 15) of 80% lean/20% fat ground beef from the same production lot and day was fabricated into 151.2 g (approximately 13 cm diameter; 1 cm thick) patties utilizing a commercial patty former. Each chub was randomly assigned to one consumer panel session and pairs of patties were randomly assigned to 1 of 8 different labeling terms: all natural (**AN**), animal raised without added antibiotics (**WA**), animal raised without added hormones (**WH**), fresh never frozen (**FF**), grass-fed (**GF**), locally sourced (**LS**), premium quality (**PQ**), and USDA organic (**OR**) plus an unlabeled sample (**NONE**) to be used for consumer sensory analysis. Consumers (*N* = 105) evaluated each sample for juiciness, tenderness, texture, flavor liking, and overall liking on 0-to-100-point continuous line scales. Additionally, each trait was rated as either acceptable or unacceptable, and consumers were asked to give their purchasing intent for each sample on a continuous line scale. Despite all samples being the same, prior to sample evaluation, consumers were provided the additional labeling information on the samples with the NONE sample having no information provided. Finally, the change in ratings for each trait was calculated for consumer data compared to the NONE sample.

**Results:** There were no differences (P > 0.05) for tenderness, juiciness, texture liking, and overall liking ratings among the 9 different treatments. For flavor liking, consumers rated WA lower (P < 0.05) than AN, GF, LS, and OR, but similar (P> 0.05) to WH, FF, PQ, and NONE. Also, consumers were less likely (P < 0.05) to purchase WA compared to AN, OR, FF, GF, LS, and OR. When evaluating the change in the ratings due to labeling, for consumers flavor liking scores, PQ had the lowest (P < 0.05) percentage change compared to AN, GF, LS, and OR, while similar (P > 0.05) to the other treatments. Additionally, for overall liking all treatments increased (P < 0.05) in ratings when provided labeling terms. LS had increased (P < 0.05) ratings across all palatability traits as well as purchasing intent. No differences (P > 0.05) were found for the percentage of samples rated as acceptable for tenderness, flavor, and texture among labeling treatments. WA had a lower (P < 0.05) percentage of samples rated as acceptable for juiciness when compared to AN, WH, FF, LS, PQ, and OR. WA also had a lower (P < 0.05) percentage of samples rated acceptable overall when compared to AN, WH, FF, GF, LS, PQ, and OR, but were similar (P > 0.05) to NONE. Moreover, when evaluating the change in the percentage of samples rated as acceptable overall, disclosing WA to consumers resulted in a decrease (P < 0.05) in the percentage of samples rated as acceptable. Also, WA had the lowest (P < 0.05) change in the percentage of samples rated as acceptable for juiciness when labeling information was given when compared to AN, WH, FF, LS, PQ, and OR. **Conclusion:** Adding additional labeling claims onto the packages of ground beef is meant to increase the overall appeal of the product to consumers. All labeling terms and claims in the current study saw a "brand lift" for palatability traits when the information was provided to the consumer prior to sample evaluation.

Keywords: beef, consumer, ground beef, Labeling, palatability



#### 9

#### EFFECTS OF COOKING METHOD ON VOLATILE FLAVOR COMPOUNDS OF BEEF STRIP LOIN STEAKS

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**Objectives:** Cooking variables like heat transfer, cooking time, and others may produce different flavor compounds profile. Differences in consumer flavor perception can be explained by different concentrations of desirables and undesirable flavor compounds. Volatile compound collection and gas chromatography-mass spectrometry (GC–MS) was performed to evaluate the effect of four different dry cooking methods on the production of flavor compounds and contrasted with previous consumer panel results.

Materials and Methods: 48 Beef strip loins were selected representing four quality grades [Prime, Top (upper 2/3) Choice, Low (lower 1/3) Choice, and Select]. Four 2.54-cm steaks were obtained from each striploin and randomly cooked on four different cooking methods: electric clamshell grill (CLAM); flat top gas grill (FLAT), Charbroiler gas grill (CHAR), or Salamander gas broiler (SAL). n=12 steaks for each treatment combination. After cooking, longissimus lumborum pieces were submerged into liquid nitrogen for 30 s and the frozen meat pieces were ground for 20 s using a food processor. Each powdered sample was packed, labeled, and stored at -80°C. Subsequently, 5 g of the powder were placed into a 15-mL clear glass vial, agitated at 65°C for a 5-min in a Gerstel agitator (500 rpm). Then an 85-µm film thickness carboxen polydimethylsiloxane (CAR/PDMS) solid-phase microextraction (SPME) fiber was exposed in the headspace above the sample for 10 min. Following a 10- min extraction period, the SPME fiber apparatus was capped with a septum (LB-2, Supelco) and volatile compound spectra were obtained. Flavor compound concentrations were analyzed using the GLIMMIX procedure of SAS (vers. 9.4), the experimental design was as split-plot with USDA quality grade as a whole plot factor, the strip loin as the whole plot unit, and the cooking method as a subplot factor. **Results:** Flavor compounds were selected and quantified (ng/g cooked sample). There were no interactions between the cooking method and quality grade for any of the selected flavor compounds (P > 0.05). Pyrazines are powerful flavor compounds derived from Maillard reaction pathways. Steaks cooked on CHAR had the greatest (P < 0.05) concentration of pyrazines followed by FLAT, then for SAL, and the cooking method with the lowest pyrazines concentration was CLAM. Aldehydes and alcohols are usually associated with rancid off-flavors. CHAR presented a lower concentration of aldehydes like pentanal and heptanal, and a lower concentration of the alcohol 1-hexanol. On the other hand, CLAM presented a higher concentration of 1-hexanol and pentanal; however, the concentration of 1-hexanol did not differ (P >0.05) from SAL and FLAT. In the previous consumer panel study, CHAR presented higher (P < 0.05) flavor scores than FLAT and CLAM but it did not differ from SAL.

**Conclusion:** Different cooking conditions evaluated allowed the formation of different flavor compounds. CHAR produced more pyrazines flavor compounds known to contribute to roasted, grilled, and nutty characteristics (Mottram, 1991). On the other hand, Clam presented the lowest concentration of Pyrazines and the highest concentration of some aldehydes and alcohols usually associated with off-flavors.

Keywords: cooking method, Volatile flavor compounds



10

### STUDENT PERFORMANCE USING A DIGITAL MEAT SCIENCE TEXTBOOK CORRELATES WITH STUDENT PERFORMANCE IN IN-PERSON INSTRUCTION

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**Objectives:** The use of digital teaching technologies has increased dramatically in recent years. Digital textbooks can allow students to access course materials across multiple devices while providing instructors new opportunities to evaluate student learning. However, questions remain as to how student performance using digital textbooks relates to student performance when course content is delivered with in-person instruction. Therefore, the objective of this study was to determine whether correlations existed between digital textbook and in-class student performance for meat science lecture and laboratory courses.

**Materials and Methods:** A digital textbook (<u>Meat Science: Conception to Consumption</u>; Zuelly, 2020) was added to the required course materials for ANSC 35100 (lecture, n = 159 students) Spring 2020, and ANSC 35101 (laboratory, n = 62 students) Spring 2021 at Purdue University. Chapter topics were: muscle biology, conversion of muscle to meat, meat quality, meat grading, carcass fabrication, processed meats, food safety, and careers in meat science. Each chapter ended with a quiz (TB Quiz) consisting of closed-ended questions, and a critical thinking assignment (TB Assign) consisting of open-ended question(s) related to the chapter materials. TB Quiz and TB Assign were combined into a textbook total score (TB Total). For in-person lecture, students were required to complete four exams (Lec Exam), daily quizzes (Lec Quiz), and two assignments (Lec Assign), that were combined for a total lecture score (Lec Total). For in-person laboratory, students were required to complete weekly quizzes (Lab Quiz), one assignment (Lab Assign), and one exam (Lab Exam), that were combined for a total lab score (Lab Total). For the purpose of this analysis, all textbook data were removed from Lec and Lab scores in order to determine the correlations between textbook and in-class scores using PROC CORR in SAS (SAS 9.4).

**Results:** For lecture, positive correlations were found between TB Total and Lec Total (R = 0.7246), Lec Exam (R = 0.6660), Lec Quiz (R = 0.7323), and Lec Assign (R = 0.3870), all with P < 0.001. When evaluated separately, TB Quiz and TB Assign were similar in relationship to all lecture score categories. For laboratory, positive correlations were found between TB Total and Lab Total (R = 0.6215), Lab Exam (R = 0.2792), Lab Quiz (R = 0.6603), and Lab Assign (R = 0.6316), all with P < 0.001. When evaluated separately, TB Quiz and TB Assign, were similar in relationship for Lab Total, Lab Quiz, and Lab Assign, but TB Quiz had stronger correlation to Lab Exam (R = 0.4605) than TB Assign to Lab Exam (R = 0.1991). **Conclusion:** These data indicate student performance with this digital textbook correlates with in-person lecture and laboratory student performance of these course. The correlation was stronger for lecture indicating that the use of the digital textbook as a wholesale replacement for in-person laboratory activities may be less impactful. While these data indicate correlations in performance between the two delivery methods, further analysis, such as qualitatively student responses to surveys and guided reflections, would be needed to determine if students perceived the use of the digital textbook enhanced their learning of the course materials.

Keywords: digital textbook, laboratory course, lecture course, student performance



#### 11

#### IMPACT OF THE COVID-19 PANDEMIC ON INSTRUCTION IN ANIMAL AND FOOD SCIENCE COURSES

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**Objectives:** The current pandemic, to an extent, has changed normal student learning. Although educational systems have been resilient, the current situation has put stress on students and instructors. The objective of the study was to discuss the effect of the pandemic on instruction in animal and food science courses.

**Materials and Methods:** All methodologies were approved by the Institutional Review Board. Eighteen questions were included in the survey instrument to assess the pandemic's impact on instruction. The questions included in the survey were related to the type of courses taught, technology used, student engagement, and grade distribution. Qualtrics was used as a survey tool, and the survey link was sent out to animal and food science faculty from several universities (primarily meat science faculty). If different formats of teaching and technologies were used, the survey allowed a faculty to record multiple responses.

**Results:** Forty-six responses were received. Prior to the pandemic, 91% of courses were taught in-person and 9% of courses taught virtually. However, during the pandemic, 26.4% in-person, 34.7% virtual, and 38.9% hybrid course formats were taught. Thirty-four percent of faculty taught small (< 25 students), 43% medium (26-99 students), and 22% large (> 100 students) class sizes. Forty-five percent taught lecture format, 8% (only laboratory), and 47% had both laboratory and lecture. Forty percent of faculty used Zoom, and 38% used university online classroom tools in teaching. However, 3.2% of faculty did not use any form of technology in teaching during the pandemic. Compared with the pre-pandemic period, 95% of faculty agreed that teaching was disrupted, 97% noted differences in student engagement, and 64% agreed there were differences in grade distribution. Sixty-one percent of faculty agreed that they had less familiarity with digital tools for teaching before the pandemic. Interestingly, 70% of faculty agreed that they currently feel more comfortable teaching with technology, and fifty-six percent of faculty plan to continue to use technology post-pandemic. Eighty percent of faculty made changes in the syllabus during the pandemic to accommodate changes in teaching. **Conclusion:** The survey results indicate that there were significant teaching adjustments to accommodate changes with pandemic and federal health guidelines. However, some of the positive outcomes, such as the use of technology and recorded lectures, may provide additional teaching options post-pandemic.

Keywords: COVID-19, pandemic, student learning, classroom technology, student engagement



#### 12

#### DETERMINING THE IMPACT OF INTRODUCTORY MEAT SCIENCE EXTENSION PROGRAMS FOR PORK AND POULTRY

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**Objectives:** Recent events have increased the interest of individuals to learn and experience meat animal harvest, fabrication, and processing. However, education in these areas is generally not available outside of university settings. This has created an opportunity to develop Extension activities to train Extension Educators, meat industry workers, meat animal producers, and consumers in the basic principles of meat science. Therefore, the objective of this program was to create an intensive, interactive Extension program to teach attendees in the areas of animal harvest, fabrication, processing, and food safety.

**Materials and Methods:** The pilot Extension program, Purdue University Boiler Butcher Basics, was developed for Spring, 2021. The in-person, hands-on program had one, 2-day, workshop for pork (n = 9 attendees) and one, 1-day, workshop for poultry (n = 14 attendees). At the beginning of each workshop, electronic surveys (Qualtrics XM<sup>TM</sup>) were completed using tablets (Pre-Program). Attendees were asked categorical questions related to demographics (gender, ethnicity, race, and age). For each of the following parameters, attendees were asked to score their initial knowledge of and confidence to perform: general animal harvest techniques, general carcass processing techniques, food safety techniques, and species-specific (pork or poultry) harvest and processing techniques. At the end of the program, attendees were asked the same questions related to knowledge and confidence (Post-Program). All scores were determined using a 10-point slider scale allowing one decimal point, with descriptions above the scale (0 = not knowledgeable/confident at all, 10 = extremely knowledgeable/confident). Each species program was analyzed separately, with differences in Pre-Program and Post-Program knowledge and confidence questions analyzed using the PROC t test in SAS (SAS 9.4), with significance determined at *P*<0.05.

**Results:** Attendees of the pork program had significantly increased knowledge scores in the Post-Program survey for general animal harvest techniques (P = 0.0249), food safety techniques (P = 0.0375), and pork specific harvest and processing techniques (P=0.0461), in addition to increased confidence to perform scores for food safety techniques (P = 0.0367). Attendees of the poultry program significantly increased all knowledge and confidence to perform scores in the Post-Program survey (P < 0.001). The differences between the species is likely related to differences in experience between the attendees, as pork attendees reported a far greater range in knowledge and confidence questions and qualitative information provided by the attendees suggested more practical experience.

**Conclusion:** The pilot program was designed to develop a curriculum, create resource materials, and gain feedback from participants in order to develop a permanent annual Boiler Butcher Basics program. Both pork and poultry programs were impactful at increasing attendees' reported knowledge and confidence scores, but poultry was considerably more significant. This suggests adding a quantitative question to the Pre-Program survey regarding experience would allow for improved analysis of this relationship. Furthermore, future programs will assign anonymous identifiers to attendee scores in order to strengthen the statistical model using a paired t test and long-term surveys will determine lasting impacts to workforce development.

Keywords: extension, fabrication, harvest, training



#### 13

### VIRTUAL FFA/4-H MEAT JUDGING WORKSHOP FOR STUDENTS AND INSTRUCTORS DELIVERED VIA YOUTUBE AND ZOOM

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**Objectives:** There has long been a need to provide meat judging training materials for students across the country. Extension educators and agriculture instructors often lack training and expertise in meat judging; thus, providing clinics, workshops, or other materials to train teams could grow interest in meat judging and the meat industry. Effective methods of providing meat judging material at a time when teams are preparing for local, regional, and state events is critical. A few widely known training tools are available, but instruction at designated times besides summer camps or clinics have only been offered in one or two states.

The COVID-19 pandemic caused most all learning and modes of delivery to shift to virtual. A positive from this was university academia, youth educators, and students adapted to using available resources. The objective of this virtual workshop ("Meat with Mafi") was to provide an eight-week course to students and instructors in preparation for 4-H/FFA meat judging contests.

**Materials and Methods:** The topics covered were USDA beef quality and yield grading, beef and pork carcass and cuts evaluation, fabrication and retail identification, notetaking for reasons and questions. The workshop was divided in eight sessions meeting on Zoom from February to April, concluding with a virtual contest. One week prior to each weekly virtual meeting, a YouTube video on the Meat with Mafi channel was sent to introduce the topic. The YouTube videos were recorded from PowerPoint presentations including complete instruction of the topic and practice carcasses, classes and/or cuts. The video each week was 30 minutes to 2 hours and additional videos were provided for instructions on how to mark the 4-H/FFA Scansheet (JudgingCard, #480-4), as well as a video describing beef carcass grid pricing and placing pricing classes, and a retail cuts evaluation video. The weekly virtual meetings included review of material, additional instruction, practice classes, example reasons/questions, and break-out rooms for more interaction, practice classes, and question/answer sessions. Each weekly virtual session lasted from 45 min to 2 hours and recorded with links provided to review at any time. In total, over 10 hours of YouTube video and 8 hours of virtual meetings with 60 carcasses for yield and quality grading, 55 judging classes with questions/reasons, and over 150 retail ID were provided. At the end of the workshop, students were divided in divisions based on age and previous judging experience for a virtual contest.

**Results:** The workshop had 220 participants (180 students and 40 instructors) from 11 states. Students ranged in age from 10 - 18 (13 and under = 24; 14 = 30; 15 = 55; 16 = 45; 17 and 18 = 26). Students had participated in 0 to greater than 10 previous meat judging contests. Over half the students (n = 95) had never competed and 30 had competed in 7 or more contests. After the contest, each instructor was mailed a flash drive with material for future training. Students surveyed at the conclusion said they would recommend or highly recommend the workshop and would like additional training in beef grading.

**Conclusion:** Based on positive feedback, the workshop will be offered annually and modified as needed.

Keywords: Meat Judging, Students, Virtual Training



#### 14

#### HOW MUCH WILL CELL BASED MEAT COST?

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#### **Objectives: General Objective**

The objective of this research is to create an enterprise budget for a theoretical cell-based meat production firm and determine the breakeven price in the current market.

#### **Specific Objectives**

- Use equipment and service quotes from industry leaders to determine the total input cost of production.
- Determine the optimal output level given size restrictions, harvesting scenarios, and equipment maintenance.

- Perform sensitivity analysis to determine price and output volatility and identify the best areas of production to cut costs.

**Materials and Methods:** An enterprise budget has been created using a combination of the annual costs of production and annual outputs to determine the wholesale cost per pound of production. This budget uses startup, production, employment, and transportation costs in addition to previously found (Specht, 2020) cell culture medium costs and harvesting scenarios. Bioreactor costs and cold storage construction are quoted from industry leaders in refrigeration manufacturing and assume a 10% cost of capital. Operating inputs such as water, labor, transportation, and packaging are estimated with respect to equipment use, industry averages, and location. Local sensitivity analysis is used on the cost of bioreactors, labor, and annual output to determine how individual changes impact the wholesale cost of cellbased meat.

**Results:** Assuming a 50% harvesting scenario with 10 harvests for each batch, meaning 50% of the batch is harvested every few days to optimize the production process, the annual output for 4 20,000L bioreactors is 1.2 million pounds of cell-based meat assuming constant production (Specht, 2020). Fixed costs including bioreactors, construction, insurance, computer infrastructure, and a facility lease, are 10.3 million dollars annually, or \$8.50/lb. Operating inputs, or variable costs including water, electricity, labor, packaging, transportation, and the cell culture medium are 21.25 million dollars annually, or \$17.58/lb. The total cost of production is \$26.06/lb of production.

Sensitivity analysis finds that 5%, 10%, and 20% reductions in the total output, likely attributable to broken equipment or inefficient production, corresponds with 5.25%, 12%, and 25% increases in the total wholesale cost holding all else equal. Analysis of the inputs, labor, bioreactors, and medium, finds that overall cost reduction of \$5/lb can be achieved through a 30% decrease in the cost of the bioreactors or the cell culture medium.

**Conclusion:** Given generous assumptions that include medium costs that are not yet possible, cell-based meat is expected to cost \$26/lb in a wholesale market, but it could cost thousands of dollars in the modern landscape (Risner et al., 2021). Cost reduction in the industry will come from the cell-culture medium, bioreactors, and labor reduction. Water, electricity, and building space make up a small fraction of the overall production, meaning this industry could compete with scientific breakthroughs. This industry will struggle to compete with traditional meat, where lean cuts cost around \$2/lb wholesale, and plant-based meat, where products cost less than \$5/lb wholesale, but it could have a large impact in niche markets for exotic meats.

Keywords: Alternative Meats, Cell-Based Meat, Enterprise Budget, Sensitivity Analysis



15

#### EVALUATING THE EFFECTS OF STORAGE CONDITION ON GREENHOUSE GAS FORMATION FROM GROUND MEAT

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**Objectives:** Previous research has determined greenhouse gas formation during various production phases of cattle, i.e., farm to plate. However, limited knowledge is currently available on greenhouse gas formation from discarded beef. The objective of this study was to evaluate greenhouse gas emissions, specifically carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and nitrous oxide (N<sub>2</sub>O), from aerobic and anaerobically stored ground beef *in-vitro*.

**Materials and Methods:** Fresh shoulder clods were purchased from a local beef processor (n = 4). Shoulder clods were ground, formed into loaves, placed on foam trays with absorbent pads, and overwrapped with polyvinyl chloride film. Following three days of display in a coffin style retail case, samples were stored in dark at 4 °C (4, 8, and 11 days) to simulate meat storage conditions at home. During dark storage, samples were collected on days 4, 8, and 11, and incubated 21.5 °C for 24 h ± 0.50 h in aerobic and anaerobic conditions for greenhouse gas analysis. The aerobic and anaerobic conditions simulate possible scenarios in a landfill. Aerobic samples were sealed with atmospheric oxygen, while anaerobic samples were flushed with 100% nitrogen gas in a vial. During retail display, surface color ( $a^*$  values) was measured using a HunterLab MiniScan spectrophotometer. Total plate count was determined on days 4, 8, and 11, and MALDI Biotyper was utilized to characterize bacteria. A gas chromatography connected with a headspace analyzer was utilized to determine greenhouse gases. The data were analyzed using the Mixed Procedure of SAS. There were four replications for all treatments.

**Results:** Surface color of loaves decreased (P < 0.05; day 0  $a^*$  values = 33.7 and day 3  $a^*$  values = 17.4) during retail display. Samples incubated in anaerobic conditions had a greater total plate count (P < 0.05) than aerobically incubated samples. The ground beef incubated in aerobic conditions had a greater concentration of CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O formation compared with the anaerobic condition. Dark storage time increased (P < 0.05) CO<sub>2</sub> concentration but not CH<sub>4</sub> and N<sub>2</sub>O levels. Bacterial characterization identified *Carnobacterium divergens*, *Hafnia alvei*, *Lactobacillus sakei*, *Lactobacillus sakei*, and *Yersinia enterocolitica*.

**Conclusion:** The results suggest that oxygen content and dark storage time can impact the greenhouse gas formation of ground beef products. To the best of our knowledge, the current research is the first report on greenhouse gas potential from ground beef. The study indicates the effect of discarded meat on the possible impact on the environment.

Keywords: beef color, environment, greenhouse gas



16

#### ECONOMIC LOSSES DUE TO BEEF DISCOLORATION

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**Objectives:** Consumers associate a bright-cherry red color of beef with freshness and wholesomeness. Any deviation from a bright red color leads to a discounted price or discarded meat. A previous report by Dr. Gary Smith and others in 2000 noted that the US beef industry lost \$1 billion annually due to discoloration. Since 2000, the meat industry has adopted several practices, including case-ready meat to enhance efficiency in meat merchandise. However, limited data is currently available on the economic losses due to retail beef discoloration. Further, meat discarded in retail stores results in societal loss of resources invested in producing meat and negative externalities that affect the environment. Therefore, the objective of the study was to determine economic losses, the amount of beef discarded, and natural resource wastage due to beef discoloration.

**Materials and Methods:** Fifty-two-week data of total beef sales, total beef discarded, and discounted sale value were collected from two national retail chains and one retail store. The two retail chains were located throughout US, and the one retail store was located in Southern US. All data were collected from meat store managers and front-line staff. No consumer data were collected. The US beef system life cycle parameters from published literature such as water, energy consumption, and carbon emissions along the beef production value chain were calculated to assess the impact of discarded meat on natural resources. The value chain includes feed production, cow-calf production, feedlot operations, packing, and case-ready operations. The data from three stores were modeled to calculate annual loss due to discoloration from the US retail beef sales.

**Results:** The data indicated total beef sales from two major retail chains and one retail store for the 52-week period was 1.1 billion pounds of steaks and ground beef. This amount of beef corresponds to approximately 10% of total retail sales in the US. The amount of beef discarded from two major retail chains and one retail store corresponds to 29.6 million pounds. Based on modeled data, the amount of beef discarded due to discoloration from the US retail beef corresponds to 350 million pounds. This results in an annual loss of \$2.1 billion due to discoloration in the US beef industry. The amount of beef discarded to estimate equivalent animals and associated natural resources lost. Results show that a 1% decrease in discolored meat across three retail stores could reduce natural resource and environmental impacts by 341 million L in water, 138 million mega Joules in energy, and 5.8 thousand tons of carbon dioxide equivalent emission consumed along the beef upstream value chain.

**Conclusion:** Beef discoloration is an inevitable process. The current research indicates that even with the advancement in technologies, meat discoloration during retail display can have a significant economic and environmental impact. Therefore, any novel technologies to improve meat color stability could improve beef production's sustainability and limit wastage of nutritious beef.

Keywords: beef color, economic losses, environmental impact



#### 17

### A PILOT STUDY ON CHARACTERIZATION OF HIDE MICROBIAL RESISTOMES OF ANIMALS IN THE DAIRY AND BEEF CATTLE PRODUCTION SYSTEMS

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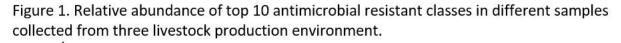
**Objectives:** Antimicrobial resistance (AMR) is an emerging public health concern. Animal hides may be contaminated by feces shedding antimicrobial resistant bacteria (ARB) directly from its host, via the interaction with other animals or the environment. The contaminated animal hide may subsequently become a potential source of ARB contamination in dairy and meat production. The objective was to characterize the resistome of animal hides and compare it to the resistome of the corresponding livestock production environment.

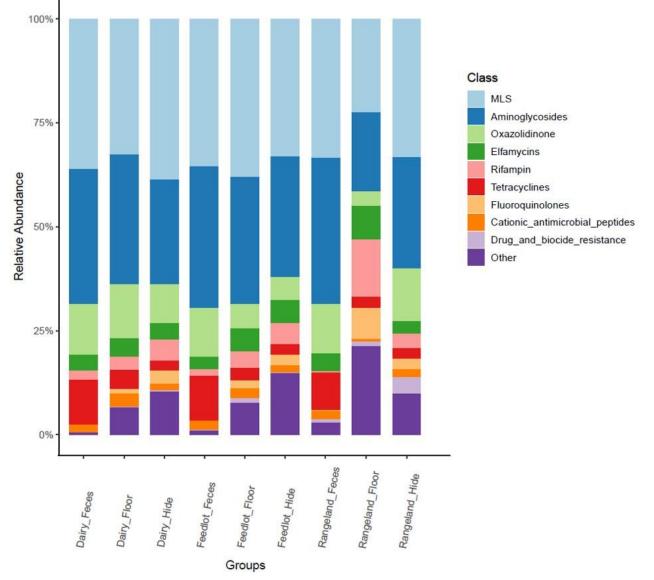
**Materials and Methods:** 36 samples were collected from three facilities, namely UC Davis Beef Cattle Feedlot, Dairy Farm and Sierra Foothill Research & Extension Center, which is a rangeland. The sample matrices include composite fecal samples, swabs from of animal hides and pen floor surfaces (4 samples/matrix/production environment). Entire microbial DNA was extracted for each sample and sequenced on Illumina NoveSeq 6000 platform. Qualified reads were aligned to the MEGARes 2.0 using Burrows-Wheeler Aligner. The relative abundance of resistome data was calculated and reported as transcripts per kilobase million. Shannon's diversity of resistomes was calculated applying vegan 2.5 in R 3.6. A one-way ANOVA was performed to compare the shannon's diversity of the resistome at AMR genes in different sample matrices within each production environment. The analysis of similarity (ANOSIM) for the resistome data at mechanism level was performed in the vegan package in R 4.1. The alpha-level was defined as 0.05.

**Results:** Across all samples, 29,890,036 reads were aligned to 916 ARGs, classified into 39 classes of resistance and 95 mechanisms. In cattle hide samples collected from the dairy, rangeland and feedlot, the most abundant three classes of antimicrobials were MLS, aminoglycosides and oxazolidinone, accounting for 73.26%, 72.72% and 67.70% of the resistomes, respectively (fig. 1). In addition, the most abundant resistance mechanism identified in the hide samples across all three facilities was macrolide-resistant 23S rRNA (MLS23S) mutation. These results were not surprising as both MLS and aminoglycosides are commonly used in dairy and beef cattle production to treat bovine respiratory diseases and mastitis. Furthermore, at the mechanism level of resistance, differences in resistome composition (ANOSIM P < 0.05) were detected in all sample matrices within each production environment. The greater resistome separation among sample matrices was detected from the dairy farm (ANOSIM R=0.67) and the rangeland (ANOSIM R=0.69), compared to those collected from the dairy farm (ANOSIM R=0.34). The results indicated that feces may not be the only source that form the resistome on animal hides. The shannon's diversity of resistomes of animal hide samples was not different (P > 0.05) from that of the rest of sample matrices in each livestock production environment.

Image:







**Conclusion:** This study characterized the resistomes of the animal hide in the selected production environments. In regard to the levels of resistomes, although the diversity was similar in hide and other samples, the composition of resistome of animal hide was still very different from those in cattle feces and pen floors within each production system. Therefore, a large-scale study is needed to better understand the formation of the hide resistome from the animal host and the environment.

Keywords: Antimicrobial Resistance, Dairy and Beef Cattle Production Systems, Resistome



#### 18.1

### TISSUE CONCENTRATIONS FROM BEEF CATTLE FED LUBABEGRON FUMARATE FOLLOWING VOLUNTARY REMOVAL FOR MULTIPLE TIMEPOINTS

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**Objectives:** Lubabegron fumarate (LUB; Experior<sup>™</sup>, Elanco Animal Health) is a feed additive approved for the reduction of ammonia gas emissions per pound of live and hot carcass weight in beef steers and heifers. A 72d study was conducted to quantify tissue residues of finishing beef cattle fed LUB.

**Materials and Methods:** Feedlot steers (n=700, 529.5 ± 8.8 kg BW) were allocated in a randomized complete block design to seven treatments within ten blocks (10 animals/pen, 10 pens/treatment) including a control (CON) and six LUB treatments followed by voluntary removal periods. LUB was fed for 56d followed by removal periods of 0, 2, 4, 6, 8 or 16d. Treatments were stagger started and harvested on a common date. Tissue samples including diaphragm, liver, rumen, reticulum, and omasum were collected from 5 animals per treatment and all were handled using commercial processing procedures. LUB concentration was determined by LC-MS/MS with a limit of quantification (LOQ) at 1  $\mu$ g/kg using parent LUB as the analytical marker. The limit of detection (LOD) was 0.4  $\mu$ g/kg for liver and 0.5  $\mu$ g/kg for all other tissues. Means were calculated using the extrapolated value for samples between the LOQ and LOD and one-half the LOD as an estimate for non-detectable (ND) samples when other samples within the treatment group were above the LOQ.

Removal Days		Liver	Muscl	Rum	Reticulum	Omas
			е	en		um
0	Mean	0.71	0.72	9.74	11.30	81.10
	Std	0.45	0.08	1.78	2.32	26.04
	Dev					
2	Mean	ND1	0.44	3.13	3.74	27.15
	Std		0.30	1.03	0.89	15.42
	Dev					
4	Mean	ND	ND	0.38	0.67	4.31
	Std			0.17	0.10	2.98
	Dev					
6	Mean	ND	ND	ND	ND	2.73
	Std					1.60
	Dev					
8	Mean	ND	ND	ND	ND	3.52
	Std					2.33
	Dev					
16	Mean	ND	ND	ND	ND	1.55
	Std					1.02
	Dev					

Table 1. Residue concentrations ( $\mu$ g/kg) of tissues from feedlot cattle fed lubabegron for 56d followed by voluntary removal periods.

<sup>1</sup>Residues are non-detectable



**Results:** All individual liver samples contained less than 2  $\mu$ g/kg LUB at 0d removal and were ND after a 2d removal. All individual diaphragm samples contained less than 1  $\mu$ g/kg LUB at 0d and were ND following a 4d voluntary removal period. Rumen and reticulum samples displayed similar residue levels to each other having declined to less than 1  $\mu$ g/kg LUB following 4d removal and became ND following a 6d removal. Omasum residue levels were higher, likely due to visible feed contamination between the folds of the organ following commercial processing. Ammonia gas emissions were calculated using the equation published by Brown et. al. (*Applied Animal* Science, 2018). Calculated cumulative ammonia gas emissions (CCAGE) over the 72d study were greater (*P*<0.001) for CON (6204 g/hd) than for LUB (5202 to 5348 g/hd). Final BW was increased (*P*<0.001) by LUB (652.7 to 658.2 kg) compared to CON (640.0 kg). Duration of removal had no impact on CCAGE (*P*>0.20) or final BW (*P*>0.20). HCW was greater by an average of 16.3 kg (*P*<0.001) when LUB was fed. HCW was 394.6, 409.6, 411.0, 413.2, 410.5, 412.8, and 408.2 kg for CON and 0, 2, 4, 6, 8, 16 d of removal treatments, respectively. The HCW response by duration of lubabegron removal was quadratic (*P*=0.042). **Conclusion:** These data indicate that tissue residue levels from cattle fed LUB are relatively low and decline rapidly following removal. Additionally, this information supports that LUB should reduce ammonia gas emissions per pound of live and hot carcass weight and that this reduction may be sustained for up to 16 days following removal.

Keywords: ammonia, body weight, feedlot cattle, lubabegron, residue



EFFECTIVENESS OF FOOD-GRADE COATING TREATED NETS AFTER VARIOUS DRYING METHODS AT CONTROLLING MITE GROWTH ON DRY CURED HAMS

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**Objectives:** *Tyrophagus putrescentiae*, known as the ham mite (Schrank), is the most difficult pest to control in the dry cured ham industry. Formerly, methyl bromide (MB) fumigation was used to control mite infestations but is classified as an ozone depleting substance; therefore, use has been limited. Food-grade propylene glycol (PG) coating treated ham nets have been successful in controlling ham mite growth and reproduction, but there are several application challenges that can be improved. Among these are the costs of shipping as wet coated nets are heavy and costly to ship; these wet nets can be inconvenient for commercial dry cured ham manufactures. The objective of this research was to determine the effectiveness of food-grade coating treated ham nets that were dried using different drying methods at controlling ham mites.

**Materials and Methods:** Polyester nets were coated with a food-grade solution (wet coated net, WN) of 40% PG, 1% propylene glycol alginate, and 1% carrageenan, using methods that were previously developed in our laboratory. Two treatments were dried on stainless steel racks (B085C6G8BM; P&P CHEF, China) in a convection oven (SCVX20E; Hobart, Chattanooga, TN) at 93.3 °C; the first for 7 min and the second for 20 min. The third treatment was dried at 25 °C on the counter in the Laboratory for 24 h. The three dried net treatments were then tested for their efficacy at controlling mites against three control treatments: uncoated polyester net (negative control), coated polyester net (positive control), and no net (negative control). The six treatments tested on the ham cubes were as follows: no net (C), coated and wet net (WN), uncoated net (CN), oven dried for 7 min (OD7m), oven dried for 20 min (OD20m), and counter dried for 24 h (CD24h). Ham cubes (2.5cm×2.5cm×2.5cm) were wrapped in the ham nets (n=5/trt) and inoculated with 20 adult mites, with the exception of the C treatment. The ham cubes were stored in ventilated jars at 25 °C and 70% relative humidity for 14 days. The total mite counts were taken on day 14.

A randomized complete block design with 2 replications was used to determine if differences existed ( $p \le 0.05$ ) among treatments. Duncan's multiple range test was used to separate treatment means when differences existed. **Results:** The OD7m (2 mites), CD24h (3 mites), and WN (2 mites) treatments had fewer mites ( $p \le 0.05$ ) than the OD20m (38 mites) and CN (45 mites) treatments. The C treatment had 183 mites, a greater number of mites ( $p \le 0.05$ ) than all other treatments. The OD20m did limit ( $p \le 0.05$ ) mite growth when compared to the C but was not as successful ( $p \le 0.05$ ) as OD7m, CD24h, or WN. This may be a result of PG evaporation. During the drying process, the OD7m and CD24h treatments lost 37.3% and 36.5% of their starting weights, respectfully, while the OD20m lost 72.0%. The greater loss ( $p \le 0.05$ ) for the OD20m likely affected the treatment's efficacy as the PG evaporated more during drying while the OD7m and CD24h retained more PG during drying.

**Conclusion:** Nets that were dried by 36-37% controlled mites but decreased product weight, which leads to easier shipping and handling by the industry. Further research could be conducted to determine the maximum weight loss that can be achieved during drying, while still effectively controlling mites.

Keywords: dry cured ham, drying, food-grade coating, ham mites, ham nets



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#### PHYSICAL EFFECTS OF CARROT FIBER AS A BINDER IN COOKED SAUSAGE

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**Objectives:** Binders and extenders are commonly used in sausage formulations to reduce costs, increase cook yield, and improve texture. Soy protein (SP) and Non-Fat Dry Milk (NFDM) are two common binders used in sausage processing. However, an increase in the amount of allergen mislabeling incidents and consumer concerns have pushed the demand for an allergen free alternative. The objective is to establish the effectiveness of carrot fiber (CF) in cooked chicken and pork sausages as an alternative to SP and NFDM.

**Materials and Methods:** Chicken breast and thigh meat were coarse ground using a 12.5-mm plate. Coarse ground meat was then added to the grinder with 1.5 L of water and a seasoning blend that consists of curing salts (A.C. Legg cure 6.25% Sodium Nitrite), sodium erythorbate, and salt. The formulation was mixed until blended and fine ground through a 9.5-mm plate. The blend was divided into seven 4.5 kg batches and randomly assigned a binder treatment: no-binder (control), CF, NFDM and SP at either 2% or 3.5% inclusion rates. Pork picnic and ham trim was processed in the same manner. Formulations were stuffed into 32-mm diameter cellulose casings and cooked to an internal temperature of 71°C, cold showered, and stored in a cooler (4°C) for 24h, vacuum packaged and frozen. Texture profile analysis (TPA) was executed in triplicate. Sausages were thawed at 5.5°C for 24h, placed in an aluminum-lined baking pan, covered in foil, and reheated in an oven at 121°C to an internal temperature of 71°C. The TPA variables (hardness, springiness, cohesiveness, chewiness, and resilience) were established using model TA.XTPlus. Statistical analysis was conducted using Statistix (Ver. 10.0 USA). Analysis was set up as a completely randomized design. Independent variable included binder treatments and dependent variables were the texture profile variables. Least square means were separated for significant main effects. Differences were detected at  $\alpha$ =0.05.

**Results:** Chicken sausages made with CF 2% and 3.5% were harder (P < 0.05) than all other treatments, and less resilient. Springiness was not different (P > 0.05) among treatments. Sausages made with CF 3.5% were less cohesive (P < 0.05) than NFDM 2%, 3.5%, SP 3.5%, and the control. Additionally, CF 3.5% was chewier (P < 0.05) than all other treatments. However, CF 2% was similar (P > 0.05) in chewiness to NFDM 3.5% and SP 3.5%. Pork sausages prepared with CF 3.5% were less springy (P < 0.05) than all other treatments. Additionally, CF 3.5% was the same (P > 0.05) as NFDM 3.5%, SP 2%, and 3.5% in hardness values. However, CF 2% was not as hard (P < 0.05) as NFDM 2%, 3.5%, and SP 3.5%. Also, CF 2% was similar (P > 0.05) in cohesiveness to SP 2%, 3.5%, and the control, with CF 3.5% as the least (P < 0.05) cohesive and resilient. Conversely, CF 2% was less chewy (P < 0.05) than NFDM 2% and 3.5%. Lastly, CF 3.5% was chewier (P < 0.05) than the control, but less chewy than NFDM 2% and 3.5%.

**Conclusion:** In conclusion, the results suggest that chicken and pork sausage made with CF 3.5% was harder and chewier than the other treatments which may not make an adequate replacement for SP or NFDM. However, CF 2% created a similar texture profile as NFDM and SP at either concentration, which may make the 2% inclusion rate of CF a more practical option for a replacement.

Keywords: Binder, Carrot Fiber, Sausage, Texture Profile Analysis



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#### TOTAL REPLACEMENT OF ANIMAL FAT IN COARSE-GROUND PORK SAUSAGES BY NOVEL BIPHASIC GELS CONTAINING HIGH-OLEIC SOYBEAN OIL

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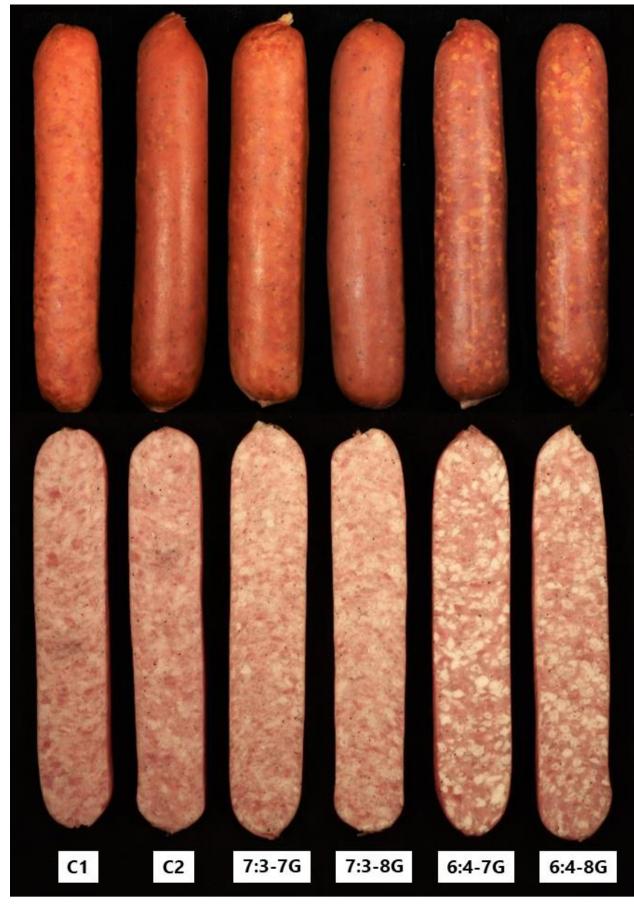
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**Objectives:** To assess the potential of a novel gelatin/rice bran wax (RBW) biphasic gel (BPG) system containing higholic soybean oil (HOSO) to replace fat in coarse-ground meat products, using smoked sausage as a model. **Materials and Methods:** BPG systems were made by combining an oleogel (OG) phase (92.5% HOSO, 7.5% RBW), and a hydrogel (HG) phase (water and either 7% (HG7) or 8% (HG8) pork skin gelatin). Control sausages were formulated to a target of 27.5% fat and were made with pork picnic and 42 trimmings **[C1]**, and lean pork knuckles (PK) and pork back fat **[C2]**. BPG treatments were made with PK and one of the following four BPGs in replacement of 100% of the pork fat: 70% OG/30% HG7 **[7:3-7G]**; 70% OG/30% HG8 **[7:3-8G]**; 60% OG/40% HG7 **[6:4-7G]**; and 60% OG/40% HG8 **[6:4-8G]**. BPG gels were ground in a meat grinder, just like the meat raw materials. Cooked sausage links were vacuum packaged, stored at 0–2°C for 98 d, and analyzed every 14 d for external and internal color (CIE L\*a\*b\*), instrumental texture (Texture Profile Analysis [TPA] and Warner-Bratzler shear force [WBSF]), and lipid oxidation (TBARS method); and on days 14, 42, 70 and 98 for sensory evaluation (10-member trained panel, 15-cm unstructured line scale). Fatty acid profile was done by GC-MS. The study was replicated three times. Statistical analysis was conducted as a mixed model using JMP Pro 15.1.0 (SAS Institute, Cary, NC), with significance set at P < .05.

**Results:** Cook/chill yield and pH were unaffected by treatment ( $P \ge .05$ ). Treatment effects on external L\* and a\* were not significant (P < .05). For internal L\*, 6:4 BPGs > 7:3 BPGs > C1/C2, with the reverse being observed for a\* (P < .05), indicating that BPGs resulted in lighter and less red products, and that an increase in the HG phase of the BPG gels increased product lightness and reduced redness. Internal b\* was highest in 7:3 BPGs and lowest in 6:4 BPGs, likely due to the yellowish hue of HOSO. Samples were lighter at d56, d70 and d98 than at d0, redder at d70 than at d0, and yellower after d0. TPA hardness was lower in C1 than 7:3 BPGs, and WBSF was lowest in C1 (P < .05). TBARS values were lower for the controls than for the BPG treatments (P < .05), and all remained under 0.90 and stable over time ( $P \ge .05$ ). For sensory attributes, C1/C2 > 7:3 BPGs > 6:4 BPGs for *Smoked Aroma* and *Smoked Flavor*, with the inverse being observed for *Off-aroma* and *Off-flavor*. *First Bite Firmness* was lowest in C1 and higher in 6:4-8G than in 7:3 BPGs. For *Creaminess* 6:4 BPGs > 7:3 BPGs. For *External Appearance* and *Internal Appearance*, 6:4 BPGs had more visible discrete particles, whereas 7:3 BPGs were not different from both controls.









**Conclusion:** We believe this study is the most successful attempt to date at mimicking animal fat in a coarse-ground meat product. Successful application of this technology will involve manipulating the mechanical properties of the BPG gels, which are determined by choice of gelators and OG:HG phase ratio. Because BPGs are lower in fat than animal adipose tissue, fat content of the final products is also reduced. Use of HOSO as the oil source of the BPGs results in a more favorable fatty acid profile. This technology also has potential in other food applications where mimicking semisolid fats is desired.

Keywords: Biphasic gel, Coarse-ground meat product, Fat replacement, High-oleic soybean oil, Sausage



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### PHYSICAL EFFECTS OF CITRUS FIBER AS A NATURAL ALTERNATIVE TO SODIUM TRIPOLYPHOSPHATE IN MARINATED BEEF

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**Objectives:** Phosphates are commonly used in marination to increase cook yields, water holding capacity, and juiciness. Clean labeling trends has resulted in preference for more natural and less processed meat products. The functionality of citrus fiber (CF) displays potential success as a phosphate replacement in processed meat. The objective of this study was to evaluate the physical effects of citrus fiber as a natural phosphate replacer in enhanced *transversus abdominis* muscle.

Materials and Methods: Beef skirt steaks (transversus abdominis) (IMPS #121D) were denuded and randomly assigned to one of five marination treatments: no phosphate (control), CF and sodium tripolyphosphate (STPP) at either 0.25% or 0.5% formulation rate. Salt level was consistent amongst all treatments, water amount varied based on formulation rate. Steaks were weighed initially, and vacuum tumbled at 15psi for 15 min, at approximately 45 RPM. Samples were weighed immediately, and 15 min after enhancement. Steaks were vacuum packaged and placed in a retail display case for 10d for retention or frozen until slice shear force (SSF) analysis. For retention, steaks were initially weighed in the bag (packaged weight), weighed after bag removal (unpackaged weight), and the bag was rinsed, dried, and weighed (bag weight). Net weight was calculated by subtracting the bag weight from the packaged weight. Purge weight was calculated by subtracting the unpackaged weight from the net weight. Purge percentage was calculated by dividing the purge weight by the steak net weight. The SSF was executed in duplicate. Steaks were thawed at 5.5°C for 24h, individually weighed, placed in an aluminum-lined baking pan, covered in foil, and cooked in an oven at 177°C to an internal temperature of 71°C. After cooling for 1h, cooked samples were weighed and cook yield percentage was calculated by dividing cooked weight by initial weight. Steaks were chilled for 18h at approximately 2 to 4°C. One cm thick, 5cm long slices were removed from each cooked steak parallel to the muscle fiber using a 45° slice box and a double blade knife. Slices were sheared once perpendicular to the muscle fibers using model TA.XTPlus. Statistical analysis was conducted using Statistix (Ver. 10.0 USA) for SSF. Analysis was set up as a completely randomized design. Least square means were separated for significant main effects. Retention data were analyzed using the PROC GLM procedure of SAS (SAS 9.4; SAS Inst., Cary, NC). Differences were detected at  $\alpha$ =0.05.

**Results:** Pickup percentage, 15 min retention and overall retention was similar (P > 0.05) among treatments. For SSF analysis, steaks marinated in CF 0.25% and 0.5% had higher (P < 0.05) shear force values compared to steaks marinated in STPP, indicating a tougher product. Also, cook yield was influenced by treatment. CF 0.25% had lower cook yield (P < 0.05) compared to STPP of either inclusion but was similar (P > 0.05) to CF 0.5%. CF 0.5% was similar (P > 0.05) to STPP 0.25% in cook yield percent.

**Conclusion:** The results suggest that beef skirt steaks marinated in CF were tougher than other treatments which may not make a suitable replacement for STPP. However, CF 0.5% yielded a similar cook yield as STPP 0.25%, which may make the 0.5% inclusion rate of CF a more practical replacement for increasing cook yield of beef products.

Keywords: beef, citrus fiber, marination, sodium triployphosphate



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COMPARISON OF LAURIC ARGINATE ESTER AND ACIDIFIED SODIUM CHLORITE AS POST-LETHALITY INTERVENTIONS IN A SLIC SYSTEM ON PROCESSED MEATS

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**Objectives:** The objectives of this study included: 1) evaluating the effectiveness of acidified sodium chlorite and lauric arginate ester on *Listeria innocua*, and 2) measuring quality characteristics of pork German sausage treated with the same antimicrobials.

Materials and Methods: Pork German sausage (NAMP# 811 Smoked Sausage) was utilized for all portions within the study. All procedures involving human subjects were approved by the ASU IRB committee (#BRA-073119). The antimicrobial treatments used in this study were acidified sodium chlorite (ASC) and lauric arginate ester (LAE). Pork German sausage links were inoculated with Listeria innocua, a non-pathogenic surrogate for Listeria monocytogenes (n = 135). The original inoculum contained 8.61 log<sub>10</sub>CFU/ml. Inoculation was performed based on the procedures of Taormina and Dorsa (2009). Five links were randomly selected and tested from each treatment (n = 15) following 2 h of storage (d 0) and plated to establish an initial bacterial count. Twenty links from each treatment were randomly assigned to d 1 and d 14 analysis (n = 60). For trained sensory analysis, non-Listeria inoculated links were randomly assigned to one of two treatments, ASC or LAE (n = 140). Two ml of each treatment was pipetted into each vacuum package following the insertion of the sausage and then vacuum sealed to mimic the Spraved Lethality in Container (SLIC) system. Sensory evaluation was conducted using a triangle test to identify which sample was different. Color of the pork German sausage was measured using a Minolta Colorimeter. These values were used to determine if any color fading occurred to the exterior portion of the sausage due to the antimicrobial applications. Data were analyzed using the MIXED models procedures of SAS. Least-squares means were computed for each dependent variable and statistically separated by a pair-wise t-test with predetermined  $\alpha$  = 0.05. Results from the triangle test were analyzed using Chisquare analysis in the Frequency Procedure of SAS.

**Results:** On d 0, sausage treated with LAE (7.18  $\log_{10}$ CFU/package) had a significantly higher concentration (P < 0.05) than both ASC (6.42  $\log_{10}$ CFU/package) and CON (6.59  $\log_{10}$ CFU/package). On d 1, the control (6.84  $\log_{10}$ CFU/package) had a significantly higher concentration (P < 0.05) when compared to the ASC (6.74  $\log_{10}$ CFU/package) and LAE (6.54  $\log_{10}$ CFU/package) treated sausage. All three treatments differed on d 14, with ASC having the lowest *Listeria innocua* concentration and the control having the highest (P < 0.05). Although sausage treated with ASC had the lowest *L. innocua* concentration between all three treatments on d 14, LAE treated sausage had the greatest log reduction. The triangle sensory test showed no significant differences between ASC and LAE with the average correctly identified samples being 32.99% and 33.68%, respectively. A statistical difference (P < 0.05) was found in the b\* value of LAE treated sausage, which was more yellow in color than those treated with ASC (37.84; 36.48, respectively). The L\* and a\* values between treatments were not significantly different.

**Conclusion:** In conclusion, ASC and LAE may both be used as post-lethality interventions in a SLIC system without negatively impacting sensory characteristic of RTE foods and processed meats in the meat industry.

Keywords: ASC, LAE, Listeria, RTE, SLIC



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MODIFIED ATMOSPHERE PACKAGING DOES NOT ADEQUATELY PRESERVE HIDES FOR GELATIN PRODUCTION G. Allen <sup>1,\*</sup>, A. Manzoor <sup>1</sup>, J. Brown <sup>1</sup>, C. Hersom <sup>1</sup>, C. Brown <sup>1</sup>, J. M. Scheffler <sup>1</sup> <sup>1</sup>Animal Sciences, UNIVERSITY OF FLORIDA, Gainesville, United States jmscheff@ufl.edu

**Objectives:** Efficient utilization of animal-byproducts is critical to both economic viability and sustainability of meat processors. Bovine hides can be further processed into leather, pharmaceutical, cosmetic, household, and industrial products. However, small processors have limited ability to supply the volume needed to be profitable. Worse, hides that are not sold must be disposed of with additional cost. Developing a method to temporarily preserve hides and allow for accumulation may help overcome this challenge. Beef hides are contaminated with microorganisms from a range of sources such as feces, dirt, water, and soil that quickly result in putrefaction of the hide. The objective of this study is to assess the efficacy of modified atmosphere packaging to temporarily preserve hides to allow for accumulation and subsequent processing into gelatin.

**Materials and Methods:** Four hundred cm<sup>2</sup> sections (20 cm x 20 cm) were cut from fresh beef hide. Sections were staked six high and placed into 3 mil polyethylene bags. Bags were randomly assigned to a sealing method including control (sealed with ambient air), vacuum flush with nitrogen, and vacuum seal. Bags were stored at 15°C with air circulation to mimic conventional storage. On days 0, 4, 7, 14, and 21, six bags per treatment were removed and a representative section was plated for total plate count enumeration. The remaining sections were unhaired with aqueous lime and thoroughly washed with water and neutralized. Sections were cut into 1 cm strips before gelatin extraction in 80°C water. Extracted gelatin was dialyzed with water for 48 h, freeze dried, then reconstituted to determine bloom strength. The study was replicated three times. Statistics were analyzed using SAS JMP; the model included day, packaging, and their interaction. Significant difference was determined at P<0.05.

**Results:** The different packaging methods showed no detectable effect on total plate count. Beef hides in all packaging resulted in an increase in total microorganisms on day 4, 7, 14, and 21, respectively compared to day 0 (P<0.001). Bloom strength progressively decreased (p=0.005) 48% from d0 to d7 and 72% by d 21 with no difference detected between treatments.

**Conclusion:** Neither vacuum packaging nor nitrogen flushing sufficiently preserve beef hides to allow for accumulation and marketing for gelatin production. Additional methods should be investigated to identify a process that allows for accumulation of hides.

Keywords: Bloom strength, Putrefaction, Vacuum packaging



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ALKALINE UNHAIRING AND PICKLING OF BOVINE HIDES FOR GELATIN EXTRACTION

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**Objectives:** Hide as a by-product of the meat industry is commonly utilized for leather production. However, supplying hides for leather processing is not economically viable for small-scale processors. Not only do small processors have difficulty marketing hides, but they also bear the cost of hide disposal. Bovine hide constitutes approximately 30% protein, and the inner corium layer is rich in collagen. Collagen is primarily used in the food, cosmetic and pharmaceutical industries and demand for collagen is increasing. Additionally, current lime unhairing methodologies take days to process and produces a waste stream with significant environmental concerns so more environmentally friendly methodologies are desired. The objective of this trial was to evaluate an alkaline unhairing and pickling method on gelatin quality.

**Materials and Methods:** Raw bovine hides were cut into approximately four hundred square centimeter patches. The hide was unhaired in 2M NaOH at a 1:1 (w/v) ratio of hide and solution for four hours at room temperature. Thereafter, patches were rinsed thoroughly with water and pickled in a 2.25M HCl solution at 1:1 (w/v) ratio of hide and solution containing different NaCl concentrations (0%, 1.5% and 2.5%) for 15 or 30 minutes under continuous stirring. Samples were washed with water and kept at 4°C overnight. For the control treatment, the hide was unhaired by soaking in aqueous lime solution for three days and no acid pickling was done. Each treatment was conducted in three replicates. Gelatin extraction was performed in a water bath at 80°C for 75 mins after cutting hide into approximately 1 cm strips. The obtained solution was filtered through cheesecloth followed by dialysis in distilled water for 48 hours. Gelatin was then freeze-dried for determination of yield, bloom strength and Hunterlab L\*, a\* and b\* color. Statistics were analyzed using SAS JMP. A Dunnett's test was used to determine difference from the lime control. Significance was determined at P<0.05.

**Results:** Compared to lime control, only pickling with 0% salt for 30 min resulted in increased (P=0.02) gelatin yield. No difference in bloom strength was detected between control and any combination of alkaline unhairing, salt concentration, and pickling time. All alkaline unhaired samples had lower (P<0.018) L\* values, higher (P<0.006) a\* values than the lime control. No salt and 1.5 % pickle resulted in higher (P<0.05) b\*values regardless of pickling time. **Conclusion:** Using 2M NaOH for dehairing and pickling in 2.25 M HCl across a range of salt concentrations (0%, 1.5% and 2.5%) for 15 or 30 mins produced gelatin of comparable bloom strength to conventional methods, albeit darker and redder in color. Further refinement of alkaline unhairing methodology may provide small processors with an option to add value to their beef hides.

Keywords: Bloom strength, Collagen, Pickling



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### ULTRASONICATION OF MEAT ENHANCES THE ACTIVITY OF SEVERAL PROTEOLYTIC SYSTEMS INVOLVED IN POSTMORTEM PROTEOLYSIS AND TENDERIZATION

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**Objectives:** Among the eating quality characteristics of cooked beef, tenderness is considered one of the most important factors dictating consumers' overall satisfaction and future purchasing decision. The development of meat tenderness has been mainly attributed to postmortem proteolysis that occurs during aging. However, the various postharvest factors that can influence proteolysis, consequently, pose a challenge for the meat industry in producing a consistently tender product. To combat this challenge, the meat industry utilized new technologies and strategies to improve the uniformity of tenderness across products. Power ultrasound is an innovative technology that offers an effective method of improving meat tenderness without negatively affecting other quality traits. At low frequency (20–100 kHz) and high intensity (< 10 W/cm<sup>2</sup>), ultrasonication is capable of disrupting biological material on both a macro and micro scale through the generation of cavitating bubbles. Such an effect is considered favorable because it can enhance proteolysis by disrupting organelles, such as the sarcoplasmic reticulum and mitochondria, which would release co-factors (i.e., calcium and cytochrome c) that can activate the calpain and caspase proteolytic systems. To the best of our knowledge, no previous studies have provided a thorough investigation on the biochemical mechanisms through which power ultrasound enhances meat tenderness. We hypothesized that power ultrasonication is capable of improving tenderness in beef steaks by causing cellular disruption and subsequently releasing available activating factors of various proteolytic systems.

**Materials and Methods:** To test our hypothesis, post-rigor (24 h postmortem) *longissimus* steaks (n=8/animal) collected from eight steers (~ 24 months old, 470 ± 11.3 kg live weight) were subjected to power ultrasonication (treatment) of 40 kHz and 12 W/cm<sup>2</sup> for 40 min at 4 °C or no ultrasonication (control). Warner-Bratzler shear force (WBSF) values, proteolysis, free calcium concentration, pH, calpain-1 autolysis, caspase-3 and cathepsin B activity were evaluated at 0, 24, 168, and 336 h post-treatment. In addition, isolated mitochondria were collected at each time point in order to evaluate mitochondrial efficiency and respiration. Collected data were analyzed using a Student's t multiple comparison test, with P ≤ 0.05 considered statistically significant.

**Results:** Our results showed that ultrasonicated steaks had lower (P < 0.05) WBSF values than controls at each time point. In conjunction with the increase in tenderness, increased (P < 0.05) troponin-T degradation at 24 h and desmin degraded at 168 and 336 h was observed in the treated steaks. Ultrasonicated steaks had greater (P < 0.05) calpain-1 autolysis immediately after treatment as well as greater (P < 0.05) free calcium concentration at 0, 24, 168, and 336 h. Additionally, an increase of caspase-3 and cathepsin B activity was observed at 0 and 168 h, respectively, in the ultrasonicated steaks. Evaluation of mitochondrial efficiency and respiration in the treated steaks indicated a significant decrease (P < 0.05) in respiratory capacity at 0 and 24 h.

**Conclusion:** Collectively, these results suggest that improvement in tenderness through ultrasonication is governed by a multitude of cellular mechanisms that involve the calpain, caspase, and cathepsin systems.

Keywords: mitochondria, postmortem proteolysis, ultrasonication



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COLOR VARIATION OF PET TREATS GENERATED FROM BEEF PROCESSING CO-PRODUCTS

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**Objectives:** Beef processing generates co-products such as liver and heart. Although these products possess a significant amount of protein and nutrients, they are often undervalued. Despite the low consumer acceptance for their own meals, beef liver (BL) and beef heart (BH) offer great potential for the development of pet treats, using ALGIN (sodium alginate and encapsulated calcium lactate) as a structure forming agent. Characteristics such as color are among the contributing factors to desirable organoleptic properties that drive consumers to purchase pet foods and treats. An experiment was designed to evaluate color variation (CV) of raw and dehydrated pet treats designed from BL and BH mixtures with ALGIN inclusion for 7 d post-production.

**Materials and Methods:** Organ meats were ground and mixed to achieve the following 3 ratios: 25% BL:75% BH, 50% BL:50% BH, and 75% BL:25% BH. Subsamples of the 3 BL:BH mixtures were combined with 2 concentrations of ALGIN (0.5% of sodium alginate + 0.425% encapsulated calcium lactate, and 1% of sodium alginate + 0.85% encapsulated calcium lactate) to produce 6 treatments. Each treatment was then extruded to generate 20-mm-thick slices, wrapped, and refrigerated at 3°C for 48 h. Squares (n = 10) of 38 × 38 mm were selected from each treatment for CV measurements on raw samples. For cooked sample analysis, raw treats were dehydrated at 93°C for 2.5 h. Raw and dehydrated pet treat CV (N=10 replicate samples per treatment) was measured by the CIE L\*, a\*, b\* color space using a Minolta colorimeter on the top cut surface after 0, 3, 5, and 7 d of storage. Data were analyzed using the GLIMMIX procedure of SAS (v 9.4) and means were separated using PDIFF at  $P \le 0.05$ .

**Results:** Interactions were observed between organ meat proportions and ALGIN concentrations. Lightness of raw pet treats was not affected by ALGIN concentration on d 0 (P = 0.1469), d 3 (P = 0.2873), and d 7 (P = 1.000), but slightly increased with increasing ALGIN concentration on day 5 (P = 0.005). However, samples became darker as BL proportions increased on d 0, 3, 5, and 7 (P < 0.0001). BL and BH combinations did not affect redness of raw treats on day 0 (P = 0.1458), and day 7 (P = 0.1053), but redness decreased as BL proportions increased on d 3 (P = 0.001) and 5 (P < 0.0001). Yellowness of raw pet foods increased as ALGIN concentration increased on d 0 (P < 0.0001), 3 (P = 0.0002), 5 (P = 0.0068), and 7 (P = 0.0079). Yellowness decreased as BL proportions increased on d 0, 3, and 5 (P < 0.0001), but no difference was observed on d 7 (P = 0.100). Cooked samples were lighter when ALGIN concentration increased (P < 0.0001) but displayed darker appearances as BL proportions increased on d 0 (P = 0.0017), 3 (P = 0.0007), 5 and 7 (P < 0.0001). Redness of dehydrated treats increased with increasing BL proportions (P < 0.0001). Yellowness of dehydrated samples increased on d 3 (P = 0.0456), 5, and 7 (P < 0.0001). Yellowness was not affected by BL and BH combinations on d 0 (P = 0.1687) and 7 (P = 0.0906) but increased with decreasing BH proportions on d 3 (P = 0.0158) and 5 (P < 0.0001).

**Conclusion:** These data suggest that liver, which liquifies when ground, can be stabilized for use in product design through the inclusion of ALGIN. ALGIN inclusion may allow processors to increase the value of organ meats when directed to a pet food market.

Keywords: alginate, Beef processing, color, co-products, pet food



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### APPLICATION OF FOOD GRADE COATINGS TO PREVENT MITE INFESTION IN AMERICAN COUNTRY CURED HAM PROCESSING

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**Objectives:** *Tyrophagus putrescentiae* is a ubiquitous pest of stored food products that contain high percentages of fat and protein, including dry-cured hams. Methyl bromide effectively controls mites in stored food products but has been listed as an ozone-depleting substance since 1992 and only existing stocks can be utilized by the dry cured ham industry. Chitosan (CH) is generally recognized as safe (GRAS) by the Food and Drug Administration and possess potential efficacy to control mites. Therefore, the objective of this research was to evaluate if chitosan-containing food-grade alternatives can be used to control mite infestation without affecting sensory attributes of dry cured hams.

**Materials and Methods:** The food-grade coating treatments were prepared as the following: (a). 0.3% CH, (b). 0.6% CH, (c). 0.3% CH + 10% propylene glycol (PG), (d). 0.3% CH + 1% xanthan gum (XG), (e). 0.3% CH + 1% XG + 10% PG, (f). 0.3% CH + 1% carrageenan (CG) + 1% propylene glycol alginate (PGA), and (g). 0.3% CH + 1% CG + 1% PGA + 10% PG. Each coating solution was coated on ham cubes (2.54×2.54×2.54 cm<sup>3</sup>, n = 5/treatment). Each coating solution was also infused in ham nets and dry-cured ham cubes (2.54×2.54×2.54 cm<sup>3</sup>, n = 5/treatment) were wrapped in the ham nets. Twenty adult mites were inoculated onto the individual cubes that were stored in ventilated jars. The jars were then stored in an environmental chamber at 24°C and 75% relative humidity for two weeks. After two weeks of incubation, the number of mobile mites on each ham cube was counted. Difference from control tests were conducted on ham slices that were wrapped with nets infused with solutions which control mites (d, e, f, g) to evaluate their impact on the sensory attributes of the ham slice.

A randomized complete block design with three replications was utilized to evaluate the efficacy of treatments on controlling mite infestations on dry cured hams. When differences occurred (P<0.05), Duncan's new multiple range test was used to separate treatment means.

**Results:** When CH was mixed with XG (0.3% CH + 10% PG + 1% XG, and 0.3% CH + 1% XG), fewer mites (15.7 and 21.0 mites) were on the ham cubes (P<0.05) in comparison to the control (211.2 mites) when the coating solution was infused in a net. Mite counts did not differ (P>0.05) between 0.3% CH + 1% XG + 10% PG and 0.3% CH + 1% XG. This indicates that addition of CH can reduce the amount of PG that is needed from 15% (previous research) to 10% in current research. Results also indicate that CH enhance the efficacy of 1% XG to control mites since 1% XG alone reduced mite growth but did not control mites to initial inoculation level from previous research. In conclusion, including CH in the coating may improve mite controlling efficacy when combining with polysaccharides such as XG. Difference from control test results indicated that no sensory differences existed (P>0.05) between CH-treated and untreated ham slices. On average, none of the CH-treated ham slices was rated above 2 on a 4-point scale where 0 = no difference, 2= moderate difference, and 4 = very large difference.

**Conclusion:** Addition of chitosan coated nets helped control mite growth when used in conjunction with xanthan gum and propylene glycol. Therefore, these coating solutions may be usable as part of an integrated pest management plan for ham producers and scale up to control mites in their aging houses.

Keywords: chitosan, dry-cured ham, food-grade, ham mites



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**RESIDUAL NITRITE AS A BASELINE FOR AN AMINO ACID ALTERNATIVE CURING SYSTEM** 

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**Objectives:** To assess the ability of L-arginine-HCL to generate residual nitrite (RNO<sub>2</sub>) values comparable to sodium nitrite (NaNO<sub>2</sub>) treated beef, pork and poultry samples.

**Materials and Methods:** Individual batches of ground beef and pork (80/20) and poultry (97/3) were mixed with 2% salt and 10% water (N=9), then split into 454 g batches. Each test batch was blended with Prague powder (6.25% NaNO<sub>2</sub>) to achieve 120, 156, or 200 ppm sodium nitrite or 1000, 2000, 3000, 4000, or 5000 ppm L-arginine with 547 ppm sodium erythorbate. After mixing (1 min) 25 g samples were extruded into two 50 mL centrifuge tubes. Sample tubes for a single species and ingredient (NaNO<sub>2</sub> N= 18; or L-arginine N=30) were placed in a controlled water bath and cooked to either 55.6, 70.0, or 73.9°C. Samples were chilled (2°C), then stored (2°C) for seven days. Residual nitrite (RNO<sub>2</sub> by UV/VIS spectrophotometry) and nitrosylation (determination of nitrosylhemochromagen (NO-H) formation) were analyzed after seven days. The experiment was a factorial (3 NaNO<sub>2</sub> or 5 L-arginine concentrations (C) and 3 temperatures (T) with two samples per concentration) randomized complete block design. This resulted in 18 (NaNO<sub>2</sub>) or 30 (L-arginine) sample tubes per cook batch for each species type, then replicated three times. Least squares means were generated and Tukey's HSD used with a predetermined significance of P<0.05.

**Results:** For NaNO<sub>2</sub> beef and pork samples, a C x T (P<0.05) interaction indicated that RNO<sub>2</sub> tended to decrease as temperature increased at each NaNO<sub>2</sub> concentration except for pork samples at 73.9°C. Poultry sample RNO<sub>2</sub> values increased as NaNO<sub>2</sub> concentration (P<0.001) increased and decreased as temperature (P<0.05) increased. L-arginine treated beef sample RNO<sub>2</sub> values decreased as temperature (P<0.0001) increased. As L-arginine concentration (P<0.05) increased, pork samples exhibited a decrease in RNO<sub>2</sub> values except at 4000 ppm. A C x T interaction (P<0.0001) was observed for L-arginine poultry samples. As concentration increased, RNO<sub>2</sub> decreased at 55.6°C. At higher temperatures, 3000 and 4000 ppm L-arginine samples tended to have lower RNO<sub>2</sub> values. All L-arginine concentrations and endpoint temperatures for each species generated similar, but generally lower RNO<sub>2</sub> values compared to NaNO<sub>2</sub> concentration for poultry samples indicated that as NaNO<sub>2</sub> concentration and temperature increased, NO-H values decreased with higher NO-H values observed at lower endpoint temperatures (55.6°C). For L-arginine treated beef, pork and poultry samples a C x T interaction indicated that as concentration increased, NO-H values tended to increase at lower endpoint temperatures (55.6°C).

**Conclusion:** L-arginine concentrations of 1000-4000 (beef), 1000-3000 (pork) and 1000-2000 ppm (poultry) generated RNO<sub>2</sub> values comparable to NaNO<sub>2</sub> treated samples at concentrations of 120, 156 and 200 ppm. Higher endpoint temperatures tended to decrease RNO<sub>2</sub> levels. Using RNO<sub>2</sub> as a baseline L-arginine concentration generated similar RNO<sub>2</sub> values compared to samples treated with 120, 156 or 200 ppm NaNO<sub>2</sub> at different endpoint temperatures. This indicates L-arginine has potential to be an alternative curing system in processed meat products.

Keywords: cured meats, L-arginine, nitric oxide, sodium nitrite



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MOISTURE UPTAKE IN BEEF JERKY AS IMPACTED BY RELATIVE HUMIDITY AND ASSOCIATED MOLDING

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**Objectives:** This study evaluates the ability of beef jerky to uptake moisture from a high relative humidity (RH) environment, and the ability of Verdad<sup>®</sup> Powder N30 (cultured cane sugar; CCS) to prevent mold growth in elevated RH conditions.

#### Materials and Methods: Experiment 1

Beef *Semitendinosus* were denuded, sliced 1.3 cm. thick, and further portioned to 1.2 cm × 1.6 cm pieces. Samples were vacuum tumbled with a brine containing 2.60% salt, 9.4% sugar, and 100 ppm ingoing sodium nitrite, targeting 17% extension. Samples were dried in a smokehouse using a stage cook schedule to an  $a_w$  of 0.76. After reaching target water activity, samples were vacuum packaged and cook yield, pH,  $a_w$ , and moisture were reported. Samples were placed into a sealed environment where the RH was modified using two-way moisture packets to maintain a target RH of 80% ± 3%, 70% ± 3%, or ambient (no two-way moisture packets). The RH was monitored throughout the study using a hygrometer and the water activity was measured every 7 days for 28 days. A single replication was performed to monitor moisture uptake.

#### Experiment 2

Samples were prepared similar to experiment 1 using a formula using a brine containing 1.65% salt, 6.0% sugar, 100 ppm sodium nitrite, and a treatment consisting of a negative control (no antimicrobial), 0.5% CCS, or 0.75% CCS. Samples were dried in a smokehouse using a stage cook schedule to a target  $a_w$  of 0.81 ± 0.02. Finished samples from each treatment were assigned to either an inoculated on non-inoculated group. Inoculated samples were spread with 1000 spores per sample from a cocktail containing *Penicillium roqueforti, Penicillium commune, Penicillium crustosum, Penicillium chrysogenum, and Aspergillus flavous.* Both inoculated and non-inoculated samples were placed into a sealed environment with 80% ± 3% RH controlled via two-way moisture packs. The RH was monitored throughout the study using a hygrometer and samples were inspected for visible mold on a regular basis. Thirty pieces per treatement were monitored throughout the shelf life.

**Results:** Beef jerky samples stored at an elevated RH saw in increase in a<sub>w</sub>, % moisture, and pH between day 0 and day 28 (see table 1). Of the inoculated samples mold was observed in control samples on day 13 and 100% of samples had visual mold growth by day 42. Non-inoculated control samples began to mold on day 20, on day 72 20% of control samples had visible mold. No visual mold growth was observed in samples formulated with CCS throughout the 72-day shelf life.

Image:



	Day 0	Day 28 80% Relative Humidity	Day 28 70% Relative Humidity	Day 28 Ambient (~45% RH)
Water Activity	0.76	0.83	0.79	0.75
Moisture Content	28.89%	35.64%	32.68%	28.33%
рН	5.60	5.76	5.75	5.73

Table 1: Change in proximate values of beef jerky over 28 days of elevated RH

**Conclusion:** When beef jerky is exposed to an elevated RH environment the water activity and moisture content increase, while pH remains relatively unaffected. The changes in product characteristics mean shelf-stable beef jerky products can become susceptible to mold growth once opened. Verdad<sup>®</sup> Powder N30 can prevent mold growth from occurring in beef jerky once the package is opened by consumers.

Keywords: beef, moisture, mold, relative humidity



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EFFECT OF HIGH HYDROSTATIC PRESSURE AND VACUUM COOKING AT LOW TEMPERATURE ON THE TEXTURE AND COOKING LOSS OF LEAN BEEF PATTIES

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**Objectives:** High hydrostatic pressure processing (HPP) induces changes in meat textural properties and can be used to develop new products or improve functional properties (Tewari *et al.*, 1999). Sous-vide (SVCOOK) can enhance the tenderness of inherently tough meat cuts (Park *et al.*, 2020). We hypothesized that the combined use of HPP and SVCOOK bring about beneficial effects on textural traits and reduce cooking losses of locally produced lean beef. Therefore, the present experiment examines the effects of combining HPP with SVCOOK on texture parameters and cooking loss of lean beef patties. Two cuts of the top sirloin cap portion of *Biceps femoris* (BF) muscle were procured (3 days postmortem) from three bullocks (11 to 13 months of age) of the local Pyrenean breed certified as Ternera de Navarra (NAVEAL), a Protected Geographical Indication (PGI) in Spain, Europe.

**Materials and Methods:** The research followed the official guidelines for the humane treatment, care, and handling of animals (Directive 2010/63/EU, Council Regulation 1099/2009). Proximate analysis of the raw meat was conducted. The meat was minced at 1500 rpm for 30 seconds using a meat grinder and then pressed into 150g patties using a patty press. The patties were HPP processed and subsequently subjected to SVCOOK. A central composite design of 20 runs with HPP pressure, HPP pressurization time, and SVCOOK temperature as independent variables was used (350-600 MPa, 5-15 minutes, 55-65°C), center point was replicated six times. The Texture Profile Analysis (TPA) of the post-HPP SVCOOK patties was conducted using a TA-XT2i stable microsystems texture analyzer (Stable Micro Systems Ltd., Surrey, UK). Two-cycle 50% compression with a 30 kg load cell was used for the tests. Six consecutive readings were reported (Gómez et al., 2018). The TPA parameters such as cohesiveness, springiness, hardness, chewiness, resilience, adhesiveness was measured. The cooking loss of the samples was calculated (Murphy *et al.*, 1975).

**Results:** Moisture, protein, ash, and pH values for the raw meat samples were 75.7%, 21.6%, 1.2%, and 5.6 units, respectively. The fat content was low (1.3%), an "extra-lean" beef according to conventional claims for nutrition labeling (USDA, 2019). Significant modification in the texture values and cooking loss was observed in the treated meat patties (P<0.05). The cohesiveness exhibited a linear relationship with HPP pressure. The increase in HPP pressure and SVCOOK temperature elevated the springiness, hardness, chewiness, and resilience, whereas an increase in HPP pressurization time reduced the samples' chewiness. The rise in SVCOOK temperature increased the adhesiveness of the treated patties. It was noted that cooking loss increased with rising HPP pressure, HPP pressurization time, and SVCOOK temperature. Optimum processing parameters based on the lowest hardness and cooking loss were HPP treatment at 350 MPa for 10 minutes and subsequent SVCOOK at 55°C.

**Conclusion:** The recommended treatment with HPP+SVCOOK optimized the processing conditions. These findings extend the possibilities for preparing very lean beef patties from BF and suggest innovative product lines for the local agro-food industries using the PGI-NAVEAL low-fat meat products. This project is funded by European Union's H2020 research and



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Keywords: HIGH HYDROSTATIC PRESSURE, LEAN BEEF



# Meat and Poultry Processing, Ingredient Technology and Packaging

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### RETAIL LIGHTING AND POSTMORTEM AGING IMPACT THE FLAVOR COMPOUNDS OF VACUUM PACKAGED BEEF STEAKS

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**Objectives:** The objective of this study was to evaluate the volatile flavor compounds produced by individually vacuum packaged beef steaks displayed under light emitting diode or fluorescent light sources.

**Materials and Methods:** USDA Low Choice paired beef top sirloin butts, striploins, and tenderloins (n = 32) were collected at a commercial beef processing facility. Subprimals were aged for 7 d postmortem in the absence of light. Subprimals were fabricated into 2.54 cm-thick steaks representing the Gluteus medius (GM), Longissimus lumborum (LL), and Psoas major (PM). Steaks were packaged in rollstock vacuum packaging and aged for an additional 7 d before being randomly assigned to a lighting display of either fluorescent (FLUR) or light-emitting diode (LED) for 0, 2, 6, or 10 d. Following the assigned aging periods, steaks were frozen at -20 °C until further analysis. Gas chromatography-Mass spectrometry was used to analyze 56 compounds associated with cooked beef flavor. Data were analyzed as a split-split plot with subprimal serving as the whole plot, lighting as the sub plot, and days of age as the sub-sub plot. Peak cooked temperatures and cook loss percentages were used as covariates.

**Results:** Lighting type and display duration interacted ( $P \le 0.043$ ), showing an increase (P < 0.05) in ethyl benzene as display duration increased, with LED lighting exhibiting the highest concentration at d 10. Lipid derived volatile compounds, 1-octanol, octanal, nonanoic acid, and tetradecane were impacted the greatest by the interaction between lighting type and muscle type ( $P \le 0.046$ ), showing greater concentrations in LL steaks stored under LED lighting. Lipid oxidation and Maillard Reaction derived compounds were also most effected by the interaction between muscle type and display duration ( $P \le 0.049$ ). Lipid derived compounds, including ethanol and butanal, also increased ( $P \le 0.049$ ) showing an interaction for all muscle types as display duration increased. Strecker aldehyde concentration, specifically phenylacetaldehyde, also increased (P < 0.05) in all muscles over the 10 d display duration. However, 2-methylbutanal and benzaldehyde only increased (P < 0.05) in the GM over the display duration. Display duration as a main effect showed an increase (P < 0.05) in carboxylic acids and 2-Propanone by d 10. Strecker Aldehyde concentrations were also greater (P < 0.05) by d 10. Muscle type as a main effect impacted ( $P \le 0.019$ ) lipid derived volatiles, including aldehydes, which showed greater (P < 0.05) concentrations in GM and LL steaks compared to PM steaks. Furthermore, sulfurcontaining compounds, dimethyl sulfide and methanethiol, showed the greatest (P < 0.05) concentration in GM steaks than in LL and PM steaks. Finally, lighting type as a main effect impacted (P = 0.023) the Strecker Aldehyde, 3methylbutanal, showing much greater (P < 0.05) concentrations under FLUR lighting than under LED lighting. **Conclusion:** These results suggest that the impacts of muscle type and duration of display were greater on the production of volatile compounds than lighting type when analyzing vacuum rollstock packaged beef steaks. Nonetheless, lipid oxidation derived compounds were greater in LED displayed products. However, further research may be done to better understand the impact of lighting displays on flavor production in comparison to volatile flavor compound production.

Keywords: Aging, Gas chromatography, Lighting, Retail display, Volatile flavor compounds



# Meat and Poultry Processing, Ingredient Technology and Packaging

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### STRUCTURALLY RELATED PHENOLIC ACIDS DIFFERENTIALLY AFFECT THE GELLING BEHAVIOR OF OXIDATIVELY STRESSED PORK MYOFIBRILLAR PROTEIN

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**Objectives:** Polyphenol-rich spices and seasonings are commonly used in sausage products to enhance product flavor and oxidative stability. Previous research has shown that released phenolic compounds could interact with myofibrillar protein (MP) extracted by salt in meat processing to change the protein functionality. The objective of the present study was to compare the effects of six structurally related phenolic acids, i.e., gallic acid (GA), syringic acid (SA), coumaric acid (CMA), caffeic acid (CFA), ferulic acid (FA), and chlorogenic acid (CA), on the gelling properties of MP prepared from pork muscle.

**Materials and Methods:** Phenolic acids (60  $\mu$ mol/g protein) were mixed with 2% (w/v) MP at pH 6.2 and then exposed to a hydroxyl radical-oxidizing environment generated with 50  $\mu$ g glucose and 8  $\mu$ g glucose oxidase (per mg protein) in the presence of 10  $\mu$ M FeSO<sub>4</sub> at refrigerator temperature for up to 8 h. Changes in protein sidechain groups and derivatives (sulfhydryls, free amines, and carbonyls), conformation (tryptophan fluorescence), rheological properties, and gelling potential were analyzed. The phenol–MP interaction experiments were conducted with three independent trials (n = 3) on different days, each with a new batch of isolated MP. Data were subjective to ANOVA analysis, and significant differences between means were identified with the Tukey's test at *P*<0.05.

**Results:** Of the six phenolic compounds, the smallest compound, GA, caused significant loss of sulfhydryls and free amines (by 7% and 26%, respectively, *P*<0.05). Other phenols except CA exhibited no effect on the amino acid side chain groups but all quenched tryptophan fluorescence suggesting protein conformational changes. The largest molecule, CA, was the most effective which deceased the fluorescence intensity 38% and shifted  $\lambda_{max}$  from 338 nm to 350 nm. During thermal gelation, the GA-modified MP displayed the strongest interpeptide cross-linking. The elasticity (G') and breaking strength of the MP gels were markedly enhanced by the addition of phenolic acids (*P*<0.05) with the final G' value ranked in the order of GA>CA>FA>CMA>SA>CFA>control. The rate of protein thermal aggregation was linked to physicochemical modifications of MP, which affected its gelling potential.

**Conclusion:** Smaller phenolic acids with less structural hinderance and more reducing potential promoted protein crosslinking and gel matrix formation, while larger molecules with more substituent groups enhanced protein unfolding and aggregation rate. The findings suggest that variations in phenolic acid composition are a possible cause for texture inconsistency observed between meat products incorporated with different spices.

Keywords: Gelation, Myofibrillar protein, Oxidation, Polyphenols, Pork



# Meat and Poultry Processing, Ingredient Technology and Packaging

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EVALUATION OF AN AMINO ACID BASED ALTERNATIVE CURING SYSTEM ON THE PHYSIOCHEMICAL AND SHELF-LIFE ATTRIBUTES OF BEEF FRANKFURTERS

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**Objectives:** The objective of this study was to evaluate the efficacy of an amino acid-based "no added sodium nitrite" alternative curing system compared to a conventional curing system (direct addition of sodium nitrite). **Materials and Methods:** Beef (90/10) was used to create frankfurter emulsions (~13 kg) containing salt (1.8%), sodium tripolyphosphate (3500 ppm), seasonings, added water (10%) containing either 156 ppm sodium nitrite (6.25% Prague powder, conventional curing) or one of three concentrations of the semi-essential amino acid L-arginine (3000, 4000 and 5000 ppm). Frankfurter emulsions were stuffed into 26 mm cellulosic casings thermally processed using a standard frankfurter schedule until an internal temperature of 72.2°C was reached then chilled (4.4°C) peeled and vacuum packaged. The frankfurters were stored under refrigeration (2°C) until analyzed at 1, 14, 28 and 56 days post manufacture and were evaluated for proximate composition, pH, water activity, residual nitrite, color (internal and external), lipid oxidation, aerobic plate counts and volatile compounds. The study was designed as a factorial randomized complete block design with three replications. Data was analyzed using the SAS JMP Pro 15 software (SAS Institute, Inc., Cary, NC). Least Square Means and ANOVA with *P*=0.05 were used to determine significant main effects. Significant differences were determined by Tukey's HSD (*P*<0.05).

**Results:** A concentration x day interaction (*P*<0.001) was observed for residual nitrite values. Treatment frankfurters were lower in residual nitrite than the nitrite control across all L-arginine concentrations at day 1 and 7 of refrigerated storage. From day 14 to 56 days of storage, residual nitrite values for L-arginine treated frankfurters were similar to (Day 14) or slightly higher (Day 28 and 56) than. A concentration x day interaction (*P*<0.05) was also observed for lipid oxidation as indicated by 2-thiobarbituric reactive substances (TBARS) values. Treatment frankfurters tended to be higher than the control throughout the 56-day storage period but TBARS value differences tended to decrease as the length of storage increased. All treatment and control frankfurters exhibited TBARS values less than 1.0 at day 56. A concentration x day effect was observed for total aerobic plate counts. Microbial growth was similar for treatment and control frankfurters through day 28 of refrigerated storage. On day 56, treatment frankfurters tended to be 0.5 to 1.0 log CFU/g higher than the sodium nitrite control. Internal product color means were also influenced by a concentration x day interaction. L\* and b\* values were not different between treatment and control frankfurters through 56 days of storage. However, internal a\* values were approximately 50% less intense than the sodium nitrite control frankfurters for all storage days.

**Conclusion:** The results from this study indicate that L-arginine can be used as a substrate to activate the nitric oxide synthase system to generate residual nitrite and nitric oxide in a similar fashion to conventionally cured (sodium nitrite) beef frankfurters. The potential exists for L-arginine to be used to generate sodium nitrite as an alternative curing system for processed meat products.

Keywords: alternative, amino acids, cured meats, sodium nitrite



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### MUSCLE-SPECIFIC IMPACTS OF FRESH BEEF TUMBLING ON MEAT QUALITY, PALATABILITY, AND PROTEOLYTIC ATTRIBUTES OF SIRLOIN MUSCLES

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**Objectives:** Muscles originating from the sirloin area of beef carcasses often exhibit intermediate tenderness. Fresh beef tumbling (without inclusion of brine solution) has recently been demonstrated to improve tenderness and possibly other eating quality attributes of beef loins. No previous studies have evaluated the impacts of fresh beef tumbling on the meat quality, palatability, and proteolytic attributes of beef sirloin muscles. The objective of this study was to evaluate the effects of fresh beef tumbling with further postmortem aging on these traits in three sirloin muscles. **Materials and Methods:** *Mm. gluteus medius* (GM), *biceps femoris* (BF), and *tensor fasciae latae* (TFL) were obtained at 5d postmortem from both sides of beef carcasses (n=16; USDA low Choice). Muscles were distributed among the tumbled (120 min of tumbling; T120) and non-tumbled control (T0) groups. Afterwards, muscles were transversely sectioned and allocated among further postmortem aging treatments (0d or 10d). Meat quality was assessed by waterholding ability and Warner-Bratzler shear force (WBSF) values. Palatability attributes such as tenderness (myofibrillar, connective tissue, overall), juiciness (overall), and flavor (beef flavor, liver-like, oxidized, and others) were determined by trained sensory panelists (n=8) at Texas Tech University. Attributes such as degradation of troponin T and desmin, collagen content and solubility, and myofibril fragmentation index (MFI) were evaluated. Data were analyzed in a balanced complete block design with a factorial arrangement of tumbling and aging treatments using the PROC GLIMMIX procedure of SAS (9.4, SAS Institute, Cary, NC).

**Results:** Fresh beef tumbling induced higher moisture losses in all three muscles for purge loss at 0d and cook loss (P<0.05) but not purge loss at 10d and thaw loss (P>0.05). Lower WBSF values were found in the T120 group for GM and TFL muscles (P<0.05) but not BF muscle (P>0.05). However, BF muscle from the T120 group at 10d further aging had less intact troponin T compared to other treatment groups within BF muscle (P<0.05). Higher MFI was found in the T120 group for all muscles compared to T0 controls (P<0.05). In general, collagen content and solubility were not affected by tumbling and aging treatments (P>0.05). Trained panelists found T120 GM muscles to have higher myofibrillar tenderness, but a slight oxidized flavor compared to T0 (P<0.05). The T120 treatment decreased juiciness of BF muscle (P<0.05), while no other sensory attributes were affected (P>0.05). Higher liver-like flavor was observed in T120 TFL muscle compared to T0 (P<0.05).

**Conclusion:** The results of this study suggest the effects of fresh beef tumbling on quality, palatability, and proteolysis would be muscle specific. Effects of tumbling were most apparent in GM muscle where lower WBSF values and higher myofibrillar tenderness were observed. While WBSF and sensory tenderness of BF muscle were not impacted in the present study, there was evidence of enhanced proteolysis through tumbling. Accordingly, optimization of the process regarding durations of tumbling and aging would be necessary to improve the quality of individual muscles from the beef sirloin region. However, as some slight detriments to trained panel sensory flavor scores were observed, further research into the impacts of fresh beef tumbling on oxidative stability would be warranted.

Keywords: beef quality, instrumental tenderness, meat tumbling, postmortem proteolysis, trained sensory panel



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MITOCHONDRIAL CALCIUM BUFFERING LIMITS POSTMORTEM PROTEOLYSIS IN AN IN VITRO MODEL

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**Objectives:** Among all meat quality attributes, tenderness is arguably the most important characteristic determining overall customer satisfaction and future intentions to repeat purchase. Despite being influenced by several intrinsic and extrinsic factors, it is widely regarded that postmortem proteolytic degradation of muscle proteins (proteolysis) is the main contributor to end-product tenderness. Proteolysis is a process that relies mainly on the calcium dependent protease, calpain-1. While a great deal of effort has been devoted to understanding factors controlling calpain-1 activity, undesirable variation in meat tenderness still exists, suggesting that the mechanisms and modulators of calpain-1 activity are not fully understood. Mitochondria are often disregarded in their involvement within postmortem proteolysis; however, recent research suggests that these organelles can sequester and store large amounts of calcium during the postmortem period. Hence, this research aims to investigate mitochondrial calcium buffering capacity and its influence on modulating calpain-1 activation during the postmortem period. Using an in vitro model, we hypothesize that mitochondria may delay the activation calpain-1 by sequestering a proportion of the available calcium. Materials and Methods: To test this hypothesis, we conducted two separate experiments. In the first experiment, mitochondrial calcium buffering capacity was evaluated in a mannitol-sucrose buffer containing different concentrations of mitochondrial protein (0, 0.5, or 2.0 mg/ml) and calcium (0, 50, or 100 μM). After 15 min of incubation, free calcium concentration was determined using an ion selective calcium electrode. In the second experiment, we sought to simulate postmortem conditions within muscle tissue for the purpose of showing mitochondrial impact on proteolysis. 0, 0.5, or 2.0 mg/ml of isolated mitochondrial protein was incorporated into an *in vitro* system containing homogenized pre-rigor bovine *longissimus* muscle in a reaction buffer containing all of the metabolites and cofactors required for postmortem metabolism. Calpain-1 autolysis and proteolysis were evaluated at 0, 120, and 240 min. Data was then analyzed using a Tukey-Kramer multiple comparison test, with  $P \le 0.05$  considered statistically significant. **Results:** Our results showed a significant decrease in calcium concentrations (P < 0.0001) proceeding the addition of mitochondrial protein. Furthermore, treatment of mitochondria resulted in a significant reduction (P < 0.05) in the degradation of troponin-T at 120 and 240 min. A trend was also observed for calpain-1 autolysis in which less autolysis occurred with increasing mitochondria concentration.

**Conclusion:** The current results suggest that mitochondria, in their capacity to buffer calcium in an *in vitro* system, has the propensity to limit postmortem proteolysis. Collectively, this research will further our understanding of biochemical processes demonstrated in postmortem proteolysis and provide evidence that mitochondria can influence meat tenderness.

Keywords: calcium buffering, mitochondria, postmortem proteolysis, tenderness



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**TRAINED SENSORY PANEL EVALUATION OF THE IMPACT OF BONE-IN VS. BONELESS CUTS ON BEEF PALATABILITY** K. J. Farmer <sup>1,\*</sup>, E. S. Beyer <sup>1</sup>, S. G. Davis <sup>1</sup>, K. M. Harr <sup>1</sup>, M. D. Chao <sup>1</sup>, J. L. Vipham <sup>1</sup>, M. D. Zumbaugh <sup>1</sup>, T. G. O'Quinn <sup>1</sup> <sup>1</sup>Animal Sciences and Industry, Kansas State University, Manhattan, United States kjfarmer@ksu.edu

**Objectives:** The objective of this study was to determine the palatability attributes of beef cuts (strip loin, tenderloin, and ribeye) of varying bone states and quality grades.

Materials and Methods: Left and right sides of 12 beef carcasses representing USDA Choice (upper 2/3) and USDA Select quality grades were selected by trained Kansas State University (KSU) personnel at a commercial abattoir in the Midwest. Paired (n = 12 pairs; 24 total/cut/grade) beef short loins and export ribs/boneless ribeye rolls were collected and transported to the KSU Meat Laboratory. Short loins were fabricated into either a boneless strip loin with a corresponding bone-in tenderloin or a bone-in strip loin with a boneless tenderloin at 3 days postmortem. In total, product was aged for 28-d and then fabricated into 2.5-cm thick steaks and frozen. Steaks designated for trained sensory analysis were thawed at 2 to 4°C for 24-h prior to cooking. Weight was measured on the raw and cooked samples as well as the edible lean and inedible bone and fat portions of the steaks to calculate cook loss and yield. Steaks were cooked to a peak temperature of 71°C on clamshell style grills. A total of 18 sensory panels were conducted at the KSU Meat Science Sensory Lab. Panelists ranked the samples on a 100-point continuous line scale with descriptive anchors at 0, 50, and 100. A sensory score of 0 corresponded to extremely dry/tough/none/extremely bland/no off-flavor; 50 neither dry nor juicy/neither tough nor tender; and 100 extremely juicy/tender/abundant/extremely intense. Data were analyzed as a split-plot design with a whole plot factor of quality grade and sub-plot factors of muscle and bone state. Results: Overall, bone status had a minimal impact on palatability traits. However, bone-in tenderloins and bone-in ribeyes were rated more flavorful (P < 0.05) than boneless cuts from the same muscle. There were no beef flavor intensity differences observed for bone-in and boneless strip steaks. Bone state had no effect (P > 0.05) on initial juiciness, myofibrillar tenderness, overall tenderness, or WBSF for any cut. Bone-in strip loin samples were rated juicier (P < 0.05) than tenderloins and boneless ribeye samples. Furthermore, tenderloin samples were ranked higher (P < 0.05)for myofibrillar and overall tenderness than strip loin and ribeye steaks which were which were rated similar (P > 0.05) by trained panelists. Moreover, there was no difference (P > 0.05) in the WBSF values for strips and ribeyes with tenderloin samples having the lowest (P < 0.05) average peak force. Lastly, USDA Choice samples were rated higher (P < 0.05) 0.05) for all palatability traits and had lower WBSF values than Select samples.

**Conclusion:** The differences observed within palatability traits show that bone-in and boneless cuts of the same muscle rate similar regardless of bone state. This provides evidence that a similar overall eating experience could be derived from a bone-in or boneless steak from the same cut and grade.

Keywords: Beef, Bone-In, Boneless, Tenderloin, Trained Sensory Analysis



#### 38

#### DESCRIPTIVE AND CONSUMER SENSORY CHARACTERIZATION OF BEEF STRIP LOINS WET AGED IN DIFFERENT TEMPERATURE ENVIRONMENTS AND DURATION

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**Objectives:** The objective of this study was to evaluate the influence of beef wet aging temperature and duration on subjective and objective measures of beef palatability.

**Materials and Methods:** Paired USDA Choice strip loins (n=60) were collected from a commercial beef processing facility. Each carcass was assigned to a storage temperature (-2, 0, 4°C). Strip loins were portioned into half loins and assigned to an aging duration (14, 28, 42, 56d). Loins were aged in commercial upright refrigerators. After aging, loins were fabricated into 2.54 cm steaks and assigned to either slice shear force (SSF), descriptive sensory analysis, or consumer sensory analysis. Steaks were cooked to reach an internal temperature of 71°C. Untrained consumers (n=200) evaluated 7 samples for flavor, juiciness, tenderness, and overall liking using a 100-point line scale where 0=extremely tough/dry/bland/dislike and 100=extremely tender, juicy, intense, like. Trained sensory panelists evaluated 16 beef flavor and texture attributes using a 100-point line scale where 0=extremely tough/dry/not detectable and 100=extremely tender/juicy/intense. All sensory data were collected using digital surveys on electronic tablets. Data were analyzed as a split-plot where loin portion served as the whole plot and aging duration served as the sub-plot. An alpha of *P*≤0.05 was used. Principal component analysis (PCA) was conducted to further understand relationships between consumer and descriptive sensory scores.

**Results:** No interaction was observed for SSF values (P>0.05). Loins aged in 4°C environments produced more tender steaks compared to -2 and 0°C (P=0.002). Steaks aged for 42 and 56 d were the most tender steaks (P<0.001). Steaks aged for 14 d were the toughest and steaks aged 28 d were intermediary (P<0.001). For descriptive sensory analysis, an interaction was observed for beef identity, bloody/serum, fat-like, liver-like, bitter, sour, and musty/earthy (P<0.05). Loins aged for 56 d at 4°C were the most intense for liver-like, sour, and musty/earthy notes compared to all other treatments (P<0.05). Umami, metallic, salty, oxidized, and overall tenderness were impacted by aging duration (P<0.05). Steaks from loins aged for 56 d had the lowest umami and salty intensity compared to all other aging durations (P<0.05). For consumer sensory analysis, an interaction was observed for juiciness, tenderness, and overall liking (P<0.05). Flavor liking was not impacted by storage temperature, aging duration, nor their interaction (P<0.05). Steaks from loins aged for 14 d at -2°C were the rated the least for juiciness, tenderness, and overall liking (P<0.05). Extended aged steaks (42 and 56 d) were rated the most tender by consumers regardless of storage temperature (P<0.001). PCA was conducted to visualize relationships between treatments and sensory attributes. Factor 1 and Factor 2 explained 43.17% and 23.49% of the variation, respectively. Off-flavors (sour, oxidized, liver-like) clustered with loins aged for 42 and 9°C were closely related to all consumer liking attributes.

**Conclusion:** Extended aging and increased storage temperatures improved tenderness. Off-flavor intensity increased during extended aging but was dependent on storage temperature.

Keywords: flavor, sensory, temperature, tenderness, wet aging



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INHIBITION OF PYRUVATE DEHYDROGENASE ACCELERATES ANAEROBIC GLYCOLYSIS IN AN IN VITRO MODEL M. J. Taylor<sup>1,\*</sup>, J. F. Buhler<sup>1</sup>, C. D. Stafford<sup>1</sup>, D. S. Dang<sup>1</sup>, S. W. El-Kadi<sup>2</sup>, D. E. Gerrard<sup>2</sup>, S. K. Matarneh<sup>1</sup> <sup>1</sup>Department of Nutrition, Dietetics, and Food Sciences, Utah State University, Logan, <sup>2</sup>Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, United States kenzietaylor4.mt@gmail.com

Objectives: The rate of postmortem pH decline is one of the most important factors involved in the development of meat quality attributes. Normally, pH gradually declines from 7.2 in living tissue to an ultimate value of approximately 5.6. This "normal" pH decline pattern is associated with most desirable meat quality attributes, whereas rapid pH decline is the immediate reason for pale, soft, and exudative (PSE) meat defect. The decline in postmortem muscle pH is driven by postmortem anaerobic metabolism, where the rate of pH decline is a reflective of the intensity of metabolism. However, the biochemical mechanisms controlling this metabolically complex event remain rather unclear. While anaerobic glycolysis is, in fact, the dominant pathway postmortem, recent studies suggest that mitochondria can influence postmortem metabolism by competing with lactate dehydrogenase for pyruvate. Thus, the ability of mitochondria to metabolize pyruvate may reduce the pyruvate pool available for anaerobic glycolysis and, subsequently, the rate of pH decline. Therefore, the objective of this study was to elucidate the influence that the mitochondria have on the rate of postmortem pH decline. We hypothesized that inhibition of pyruvate dehydrogenase (PDH) and pyruvate carboxylase (PC) provides more substrate for anaerobic glycolysis, thereby increasing the rate of pH decline. Materials and Methods: To test this hypothesis, we utilized CPI-613 and Avidin, which are known inhibitors of PDH and PC, respectively, in an *in vitro* system that is intended to mimic postmortem metabolism. Pre-rigor porcine *longissimus* muscle samples (n = 8) were collected immediately after harvest, powdered under liquid nitrogen, and homogenized in a reaction buffer containing all the constituents required for postmortem metabolism to occur. Four treatments were tested: control, CPI-613 (400 μM), Avidin (1.5 U/ml), and CPI-613 + Avidin. In a follow-up experiment, universally labeled

glucose tracer ([<sup>13</sup>C<sub>6</sub>] glucose) along with CPI-613 and Avidin were incorporated into the *in vitro* system to precisely track the fates of pyruvate using GC-MS and <sup>13</sup>C-mass isotopomer distribution analyses. Collected data were analyzed using a Tukey-Kramer multiple comparison test, with  $P \le 0.05$  considered statistically significant.

**Results:** Our results showed that samples treated with CPI-613 (with or without Avidin) had lower pH (P < 0.05) and greater lactate and glucose-6-phosphate accumulation and glycogen degradation (P < 0.05) at 60, 120 and 240 min compared to control. No treatment effect was observed in PC inhibited samples with Avidin. The results from the follow-up experiment indicated lower enrichment of tricarboxylic acid cycle intermediates (alpha-ketoglutarate, succinate, and malate; P < 0.05) in CPI-613 + Avidin treated samples compared to control.

**Conclusion:** Collectively, our data shows that inhibition of PDH increases glycolytic flux and decreases the enrichment of mitochondrial metabolites in an *in vitro* model, which suggests that mitochondria may be involved regulating postmortem glycolysis.

Keywords: mitochondria, pH decline, postmortem metabolism



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### INFLUENCE OF POSTMORTEM AGING AND STORAGE CONDITIONS ON TENDERNESS OF STRIPLOIN STEAKS FROM GRASS- AND GRAIN-FINSIHED BISON BULLS

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**Objectives:** The objectives of this study were to: 1) compare the influence of postmortem aging on tenderness of striploin steaks from grain- and grass-finished bison bulls, and 2) compare the influence of frozen storage on tenderness of striploin steaks from grain- and grass-finished bison bulls.

**Materials and Methods:** Bison bulls were randomly assigned to two finishing treatments: Grain-finished (n = 30, backgrounded on pasture and finished for 130 days with ad libitum access to grass hay, alfalfa, whole corn prior to slaughter) or Grass-finished (n = 30, remained on pasture until slaughter). Bulls were slaughtered at approximately 30 months of age. Striploins were collected from both sides of each carcass and fabricated into 2.54-cm steaks. One steak was removed from the right striploin, vacuum packaged, and stored fresh for 14 d at 4°C. Four steaks fabricated from the left striploin were aged for 4, 7, 14, or 21 days, vacuum packaged, and frozen (-20°C) for approximately 5 months. Warner-Bratzler Shear Force (WBSF) was utilized to determine objective tenderness. Frozen steaks were thawed at 4°C for 24 h before cooking and all steaks were weighed prior to cooking to an internal temperature of 71°C. After cooking, all steaks were cooled to room temperature (20°C) and reweighed to determine cook loss. Five to six 1.27-cm cores were removed from each steak and sheared once perpendicular to the muscle fiber orientation and peak force was recorded. An average shear force value was then calculated for each steak. For Objective 1, shear force and cook loss data were analyzed as repeated measures using the compound symmetry covariance structure in the MIXED procedure of SAS for effects of finishing treatment, aging, and their interaction with peak temperature included as a covariate. For Objective 2, shear force and cook loss were analyzed for the effects of storage treatment, finishing treatment and their interaction using the MIXED procedure of SAS.

**Results:** A finishing treatment by aging day interaction was observed for WBSF (P = 0.0047). Steaks from the grain-finished treatment became more tender (P < 0.0001) as aging time increased from d 4 to d 14, while WBSF of steaks from grass-finished bulls did not change (P > 0.05) from d 4 to d 14. Steaks from grass-finished bulls were more tender (P < 0.05) than grain-finished at d 4 and 7, but treatments were similar (P > 0.05) at d 14 and 21). Steaks from grass finished bison bulls had increased cook loss (P < 0.0001) compared to steaks from grain finished bison bulls, no effect of aging day or treatment by aging day interaction was observed for cook loss (P > 0.05). No storage treatment by finishing treatment interaction (P > 0.05) was observed for WBSF or cook loss. Frozen storage improved tenderness (P < 0.0001) of 14 d aged bison steaks compared to fresh storage but did not influence cook loss (P > 0.05).

**Conclusion:** Collectively, these data indicate grass and grain finished bison bulls respond differently to postmortem aging, with grass-finished bulls producing steaks that are more tender earlier in the aging period but exhibiting increased cook loss compared to steaks from grain-finished bulls. Additionally, freezing steaks from bison bulls resulted in improved tenderness compared to fresh storage without impacting cook loss.

Keywords: bison, frozen storage, grain finished, grass finished, tenderness



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#### EFFECTS OF INCREASED PORK HOT CARCASS WEIGHTS ON BIOCHEMICAL FACTORS IMPACTING TENDERNESS

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**Objectives:** A previous study by Rice et al. (2019) observed improved tenderness ratings as hot carcass weight increased for both consumer and trained sensory panelists. Therefore, the objective of this study was to investigate potential causative biochemical factors for the improved tenderness of heavier pork carcasses.

**Materials and Methods:** Details regarding the swine production procedures and loin fabrication for this study are described in detail by Lerner et al. (2020) and Rice et at. (2019). In brief, market hogs in this study were intentionally fed to heavier live weights that exceeded industry standards. At harvest, carcasses were assigned to one of four hot carcass weight groups. Carcasses were categorized as light (LT; under 111.8 kg), medium light (MLT; 111.8 to 119.1 kg), medium heavy (MHVY; 119.1 to 124.4 kg), or heavy (HVY; greater than 124.4 kg). A sub-sample of chops (*N* = 80) representing each of the weight treatments was utilized in this study. Sarcomere length of powdered samples was determined by phase-contrast microscopy. Thirty sarcomeres were measured and analyzed for each sample using ImageJ. Total collagen content was determined by measuring the hydroxyproline content in each sample, assuming that 14% of collagen is hydroxyproline by weight. Finally, immunoblotting was used to determine proteolysis of Troponin-T. The intact and degraded troponin-T bands were quantified, and the final calculation was displayed as the relative percentage of degraded Troponin-T for each sample. Data were analyzed as a completely randomized design with the fixed effect of carcass weight group and the random effect of kill day.

**Results:** Increased hot carcass weight had a significant effect on Troponin-T degradation. Chops from carcasses in the MHVY and HVY treatments showed a greater (P < 0.05) percentage of degraded Troponin-T than those chops from the MLT and LT groups, with a range of 6.50 to 10.26 percent. Sarcomere lengths were the same in all weight treatments, except the LT group which had the longest (P < 0.05) sarcomeres. Mean sarcomere lengths for all treatments were between 1.66 and 1.92 µm. No differences (P > 0.05) were observed for total collagen content across weight treatments with all collagen content falling between 3.97 and 4.65 mg of collagen per gram of muscle tissue.

**Conclusion:** In conclusion, chops from the MHVY and HVY treatments had more degradation of structural proteins. Rice et al. (2019) found that consumer and trained panelists ranked the heavier weight treatments more tender despite having the same fat percentage. Our results provide evidence that the slower chill rate of the heavier weight carcasses allows for greater postmortem enzymatic proteolysis contributing to the observed improved tenderness of heavier weight carcasses.

Keywords: Enzymatic Proteolysis, Heavy Weight Pigs, Pork, Sarcomere Length, Tenderness



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### EFFECT OF SOUS VIDE PREPARATION VERSUS TRADITIONAL PREPARATION ON CONSUMER RATINGS OF BEEF STEAKS FROM VARIOUS MUSCLES

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**Objectives:** The objective of this study was to determine the viability of sous vide to improve palatability of beef cuts. **Materials and Methods:** Chuck rolls, chuck clods, chuck under blades, inside rounds, outside rounds, and strip loins were selected from USDA Low Choice. Subprimals were wet aged at 2-4 °C in the dark for 21 d. Subprimals were fabricated into 2.54 cm steaks representing the Adductor (**AD**), Biceps femoris (**BF**), chuckeye steak (Longissimus thoracis, Complexus, and Spinalis dorsi; **CHE**), Longissimus lumborum (**LL**). Semimembranosus (**SM**), Serratus ventralis (**SV**), and Triceps brachii (**TB**). Steaks were randomly assigned to one of two cooking treatments, traditional grilling (**TRAD**) or sous vide cooking followed by finishing on a grill (**SVG**). Steaks were then packaged and frozen at -20°C. For analysis, steaks were thawed for 24 h, and the steaks designated for SVG were cooked in a circulating hot water bath at 63.5°C for 2 h to a medium-rare doneness (63°C). Immediately prior to serving, steaks were finished on a clamshell grill to a medium degree of doneness (71°C). Steaks designated for TRAD were cooked on a clamshell grill to a medium degree of doneness. Steaks were cut into steak thickness × 1 × 1 cm cubes and 2 cubes were served to each panelist. Untrained consumer panelists (*n* = 300) evaluated 7 samples for flavor liking, juiciness, tenderness, and overall liking on a 100-point line scale using tablets. Data was analyzed as a 2 × 7 factorial arrangement, with cooking method, muscle, and the interaction serving as fixed effects and panel session serving as a random effect.

**Results:** There was a method × muscle interaction for overall liking (P = 0.040). For the LL, AD, and the SV, the TRAD steaks were rated higher for overall liking (P < 0.05) compared to their SVG counterparts, with marked differences appearing in the LL. In contrast, the SVG TB was rated higher for overall liking (P < 0.05) over the TRAD TB steaks. No differences were observed between SVG and TRAD (P > 0.05) for the BF or SM steaks, which were the muscles preferred the least by consumers compared to all other muscles, regardless of cooking method (P < 0.05). No method × muscle interaction was observed for tenderness, juiciness, or flavor liking ( $P \ge 0.070$ ). For cooking method, TRAD steaks were rated higher for flavor, juiciness, and overall liking (P < 0.001). No differences were observed between cooking method (P = 0.595) for tenderness. Within the muscle treatments, TB, LL, and CHE steaks were rated higher for flavor liking (P < 0.05) compared to the SV and AD steaks. However, the BF and SM were the least flavorful (P < 0.05) according to consumers. The CHE, SV, and TB were juicier (P < 0.05) than the SM, AD, and BF. Similar to flavor liking, the BF and SM steaks were the driest and toughest steaks (P < 0.05). The CHE and LL steaks were more tender (P < 0.05) than all other treatments, with the exception of the TB, which was similar (P > 0.05) to the LL.

**Conclusion:** The results of this study indicate that the cooking method had a substantial impact on consumer ratings, suggesting that consumers will consistently choose TRAD cooking over SVG cooking under these sous vide conditions. Traditional steaks had higher palatability ratings by consumers than sous vide steaks. These results suggest, regardless of cooking method, high quality muscles will provide the consumer a quality eating experience.

Keywords: consumer, cooking method, muscle, palatability, sous vide



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#### INFLUENCE OF BEEF CARCASS CHILLING RATE ON STEAK CASE LIFE AND QUALITY TRAITS

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**Objectives:** The objective of this study was to determine the influence of beef carcass weight on carcass chilling, pH decline, color, case life and tenderness of steaks from the round, loin, rib, and chuck.

**Materials and Methods:** Twelve head of fed beef cattle were slaughtered over two days at the SDSU Meat Laboratory. Carcasses were allotted by hot carcass weight (HCW) into Heavyweight (HW; HCW = 450 + 19 kg) and Lightweight (LW; HCW =  $349 \pm 34$  kg) groups. Data logging thermometers were placed in the left side of the carcass within the round, loin, rib, and chuck to track temperature decline. A 20-cm logger was placed in the round and chuck and a 10-cm logger was placed in the loin and rib. During chilling, pH decline was measured in the Semitendinosus (ST), Longissimus lumborum (LL), Longissimus thoracis (LT), and the Serratus ventralis (SV) for 48 h. Carcass data including 12<sup>th</sup> rib fat thickness, ribeye area, marbling score, USDA Yield and Quality Grade were collected approximately 48 h postmortem. Following carcass data collection, sides were fabricated, and the ST, LL, LT, and SV were collected, weighed, and portioned into 2.54-cm steaks. One steak from each muscle was tray overwrapped with a high-oxygen permeable wrap and placed under a simulated retail display for 10 d where they were evaluated for instrumental color (Minolta L\*, a\*, b\*), sensory color score, and discoloration each day. Steaks from each muscle were aged 5, 10, 14, or 21 d for Warner-Bratzler Shear Force (WBSF) analysis. Statistical analysis was conducted using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) for the effect of treatment, time, and their interaction. Carcass data and muscle weights were analyzed as a completely randomized design. Temperature and pH decline, objective and subjective color and WBSF were analyzed as repeated measures. Slaughter date was used as a random variable. Significance was considered at P < 0.05. **Results:** Heavyweight carcasses had heavier (P < 0.05) HCW, and all selected muscle weights compared to LW carcasses. No effect of HCW or HCW x time (P > 0.05) was observed for pH decline. A HCW x time interaction was observed for temperature decline (P < 0.05) in the rib and round primals. Ribs from HW had increased temperatures (P < 0.05) for the first 25 h of chilling compared to LW but were similar for remainder of chilling period (P > 0.05). Temperature in the round was not different between treatments for first 3 h of chilling (P > 0.05), but HW had increased temperatures for remainder of chilling (P < 0.05). A HCW x time interaction was detected for WBSF (P < 0.05) and L\* values (P < 0.05) in

the *ST*. Steaks from LW carcasses were more tender (P < 0.05) than HW at d5 of aging, but were not different at d10, d14 or d21 (P > 0.05). Steaks from HW were darker (P < 0.05) throughout the display period. On d1 of retail display, HW steaks L\* value decreased (P < 0.05), whereas the LW steaks increased (P < 0.05). Steaks from the *ST* from HW carcasses had lower a\* and b\* values (P < 0.05) than LW steaks. *SV* steaks from HW carcasses had increased (darker) subjective color scores (P < 0.05) compared to LW steaks.

**Conclusion:** Heavier beef carcass weights resulted in a more prolonged temperature decline deep in the rib and round. However, only the *ST* had tougher steaks at early aging days and decreased L\*, a\* and b\* values in HW carcasses.

Keywords: beef, carcass, meat quality, temperature



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### EXTENDING THE SHELF-LIFE OF BEEF BONE-IN SHORT RIB STEAKS USING COMBINATIONS OF ACEROLA CHERRY POWDER AND ROSEMARY EXTRACT

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Objectives: Retailers in South Korea are currently limited in possible sales due to the rapid discoloration of steaks from beef bone-in short ribs resulting in a one-day retail shelf-life. Improving the shelf-life of these steaks by two days will improve salability of beef in international markets and therefore improve potential profits for producers. The objective of this study was to determine the effect of the topical application of acerola cherry powder and rosemary extract in combination on beef bone-in short rib steak shelf-life with regard to color stability and lipid oxidation. Materials and Methods: Beef bone-in short ribs (IMPS 123A; N = 18) from USDA Choice carcasses were purchased from a commercial harvest facility and aged (0°C) for 28 d post-fabrication. Following aging, 1.02 cm-thick steaks were cut (perpendicular to the rib bones) and systematically assigned to a treatment based on steak location within the subprimal. Treatments included: untreated control (C) or topically sprayed (2 ml) with a treatment of an acerola cherry powder solution (0.05% A), rosemary extract solution (0.10% R), or mixture of the acerola cherry powder and rosemary extract (M1 = 0.05% A + 0.1% R; M2 = 0.1% A + 0.1% R; M3 = 0.05% A + 0.2% R; M4 = 0.1% A + 0.2% R). Half of the steaks were assigned by location to d 0 lipid oxidation, metmyoglobin reducing activity, and oxygen consumption; the remaining steaks were assigned to 4 days of retail display followed by d 4 lipid oxidation and metmyoglobin reducing activity. Throughout retail display, steak objective and subjective color was measured once daily for 4 days. **Results:** On d 0 and d 4, untreated bone marrow was less red than all of the antioxidant treated bone marrow (P = 0.011), and bone marrow treated with M1, M2, M3, and R had less discoloration than the untreated control bone marrow (P = 0.016). Steaks treated with M2, M3, M4, and R had a brighter oxygenated lean color (scale: 1-8; bright cherry red to dark cherry red, respectively) than the untreated control steaks (P = 0.028). Oxygen consumption and metmyoglobin reducing activity did not differ between treatments (P = 0.570 and P = 0.315, respectively). Steaks treated with M4 did not increase in lipid oxidation from d 0 to d 4, and on d 4, steaks treated with M1, M3, and M4 had less lipid oxidation than the untreated control steaks (P < 0.001). There were differences between treatments (P = 0.034) in retail display fluid loss, however antioxidant treated steaks did not decrease fluid loss compared to the untreated steaks. Conclusion: Applying topical antioxidants improves shelf-life stability of beef bone-in short ribs aged for an extended period.

Keywords: antioxidants, beef, extended aging, shelf-life



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#### LEAN COLOR AND OXIDATIVE BIOMARKERS IN POST-RIGOR LONGISSIMUS MUSCLE FROM BEEF CATTLE INJECTED WITH HYDROGEN PEROXIDE

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**Objectives:** This study examined the effects of oxidative stress induced by hydrogen peroxide on lean color and oxidative biomarkers in post-rigor *longissimus* muscle from beef heifers (N = 18).

Materials and Methods: Cattle were supplemented with ground corn and soybean hulls to 310 to 456 kg of BW while grazing on cool and warm-season pastures and finished at a commercial feedlot in Iowa. Animals were blocked into three clusters based on initial biomarkers. Two treatments of either 20 mg hydrogen peroxide/kg BW (OX, n = 9) or 10 mL of saline (CON, n = 9) were equally and randomly assigned to animals within each cluster. On the day before slaughter, the OX and CON treatments were administered intravenously through the jugular vein. Samples were collected from the anterior of the longissimus lumborum muscles at 72 h postmortem. Samples were analyzed for thiobarbituric acid reactive substances (TBARS) by extracting malondialdehyde in 10% trichloroacetic acid and reacting with saturated thiobarbituric acid solution (532 nm), total antioxidant capacity (TAC) extracting antioxidants in water and reacting with radicalized ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic) acid; 734 nm], glutathione (GSH) by extracting and reducing all glutathione to GSH and reacting with DTNB (Ellman's reagent; 5,5'-dithio-bis-2-nitrobenzoic acid; 410 nm), glutathione peroxidase (GPx) by extracting enzyme in 50 mM Tris-HCl buffer, pH 7.6 with 5 mM EDTA) and being measured by the coupled indirect oxidation of GSH and NADPH (340 nm) to NADP<sup>+</sup>, and metmyoglobin reducing activity (MRA) based on the ability of muscle extract in 0.2 mM phosphate buffer, pH 5.6 to reduce horse skeletal metmyoglobin (MMb) to deoxymyoglobin (DMb; 580 nm). One steak per animal was also displayed under simulated retail conditions and repeatedly measured for reflectance spectra of 400 to 700 nm in 10-nm intervals and CIE L\*, a\*, b\* values. The L, a\*, b\*, and spectra were used to calculate hue angle, chroma, and percentages of DMb, oxymyoglobin (OMb), and MMb. Data were analyzed as a randomized complete block design with treatment as fixed effect and actual probability values were reported as a single value for a pair-wise comparison or a range of values for multiple pair-wise comparisons. Lean color data were analyzed as split-plot design in time (repeated measurement). **Results:** Although the CON had 0.23 mmol Trolox equivalence/kg more than OX (P = 0.028), no treatment effect was found for lean color or oxidative biomarkers in post-rigor muscles ( $P \ge 0.151$ ). Except for d 0, as the steaks continued to bloom, the lean color, although maintaining its shade (hue,  $P \ge 0.363$ ), had diminished redness (a<sup>\*</sup>,  $P \le 0.007$ ), color intensity (chroma,  $P \le 0.001$ ), and OMb percentage as retail display approached d 5 ( $P \le 0.115$ ). Conclusion: Although hydrogen peroxide injection decreased TAC, such an effect was minimal and did not change lean color and oxidative biomarkers.

Keywords: Meat color, Beef, Oxidative stress



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### POST-TRANSLATIONAL MODIFICATION OF MYOGLOBIN IN POST-RIGOR LONGISSIMUS LUMBORUM MUSCLE FROM BEEF CATTLE INJECTED WITH HYDROGEN PEROXIDE

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**Objectives:** This study examined the effects of oxidative stress induced by hydrogen peroxide on post-translational modification (PTM) of myoglobin in post-rigor *longissimus lumborum* muscle from beef heifers.

Materials and Methods: Cattle were supplemented with ground corn and soybean hulls to 310 to 456 kg of BW while grazing on cool and warm-season pastures and finished at a commercial feedlot in Iowa. Animals were blocked into three clusters based on the principal component analysis of initial thiobarbituric acid reactive substances (TBARS), total antioxidant capacity (TAC), and ferric-reducing antioxidant power values in blood plasma. Two treatments of either 20 mg hydrogen peroxide/kg BW (OX, n = 9) or 10 mL of saline (CON, n = 9) were equally and randomly assigned to animals within each cluster. On the day before slaughter, the OX and CON treatments were administered intravenously through the jugular vein. A 2.54-cm longissimus lumborum steak per animal was collected at 72 h postmortem. Myoglobin was purified using sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The protein bands representing myoglobin (17 kDa) were excised and were subjected to in-gel trypsin digestion. The tryptic peptides were extracted, concentrated, and subjected to nano-liquid chromatography tandem mass spectrometry (MS/MS). The MS/MS data were submitted to a local MASCOT server for protein identification against a custom database containing only beef myoglobin. The MS/MS data were searched for the following PTM: methionine oxidation; lysine acetylation; lysine and arginine methylation; serine, threonine, and tyrosine phosphorylation; 4-hydroxynonenal alkylation at histidine and lysine. Results: Overall, the number of PTM in myoglobin were more in OX (56 PTM) than in CON (47 PTM). Phosphorylation occurred on serine and threonine residues in both CON (9 PTM) and OX (9 PTM); whereas tyrosine was phosphorylated only in CON. Methylation and carboxymethylation were detected in lysine and arginine residues in both CON and OX. Acetylation was observed at 10 lysine residues in OX compared with 6 in CON. The 4-hydroxynonenal alkylation of histidine and lysine residues occurred more in OX (13 PTM) than in CON (8 PTM) myoglobin. Moreover, 4hydroxynonenal alkylation of distal histidine (His 64), responsible for heme stability, occurred only in OX myoglobin. **Conclusion:** The 4-hydroxynonenal modification of His 64 was particularly concerning because it is an indicator of myoglobin oxidation. The occurrence of PTM can compromise myoglobin redox stability and fresh beef color stability. These findings suggest that myoglobin PTM in postmortem skeletal muscles may be exploited as biomarkers of preharvest oxidative stress in beef cattle.

Keywords: Beef, Myoglobin, Oxidative stress



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#### CHARACTERIZATION AND PATHOGENESIS OF DORSAL RECUMBENCY SYNDROME ASSOCIATED WITH WOODY BREAST IN BROILER FLOCKS FROM ONTARIO, CANADA

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**Objectives:** A dorsal recumbency syndrome has been recently described in market-age broiler chickens. Affected birds are flipped onto their backs and unable to right themselves; described by some as "turtle chickens". These birds can eventually die on the farm and can represent a significant economic loss. Previous reports suggested that breast myopathies such as the woody breast, which is characterized by myodegeneration, inflammation, and scarring of the pectoral muscles, may be associated with this syndrome, as a cause of impaired wing movement (necessary for up righting the bird). In this study, we aimed to characterize the clinical, pathological, and serum chemistry changes of broiler chickens affected by dorsal recumbency.

**Materials and Methods:** Samples from 4 different commercial broiler farms were collected between March 2020 and April 2021. A total of 65 broilers (Ross 708), 33 affected and 32 unaffected, were sampled at 42 to 48 days of age. Clinical signs were recorded, serum was collected, and birds were euthanized for post-mortem analysis and histopathology. Samples from the *Pectoralis major* of 24 birds (13 affected, 11 unaffected) from 2 of the 4 visits were scored histologically to determine the presence and severity of myopathies. Out of 36 serum samples (18 affected, 18 unaffected) from those 2 visits, serum from 4 affected and 4 unaffected chickens was submitted to the Animal Health Laboratory for a full chemistry profile. All 36 samples were used to determine the level of aspartate aminotransferase (AST) and creatine kinase (CK). Student's t test and Wilcoxon rank-sum test were used to compare the amounts of serum analytes and histological scores between affected and unaffected birds (p < 0.05).

**Results:** Sampled flocks included males (3) and mixed sex (1), with a market weight of 2.8 to 4.2 kg. All recumbent birds were bright and alert, could move their legs but had difficulties moving their wings. Mild hydropericardium was observed in 34.4% (95% CI: 18.6 - 53.2) and 25.0% (95% CI: 11.5 - 43.4) of affected and unaffected birds, respectively; Right ventricular hypertrophy was observed in 12.1% (95% CI: 3.4 - 28.2) and 9.4% (95% CI: 2.0 - 25.0) of affected and unaffected birds, respectively. Recumbent birds displayed a significantly higher severity (p < 0.05) of myo-degeneration, inflammation, and accumulation of fibro-fatty tissue in the *P. major* compared to unaffected birds. The level of AST and CK of recumbent chickens was significantly higher (p < 0.05) compared to unaffected birds, suggesting muscular damage. **Conclusion:** Serum chemistry and histopathological changes in the *P. major* suggest an association between breast myopathies and dorsal recumbency syndrome. Recumbent birds showed higher frequency of hydropericardium and right ventricular hypertrophy, suggesting these lesions may lead to death.

Keywords: biochemistry, broiler chicken, cardio myopathy, histopathology, woody breast myopathy



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#### AN INVESTIGATION ON THE INFLUENCE OF VARIOUS BIOCHEMICAL TENDERNESS FACTORS ON EIGHT DIFFERENT BOVINE MUSCLES

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**Objectives:** Tenderness is defined as the amount of force required to bite through a piece of meat. Despite its simple definition, three factors underlie the complexity of tenderness: the actomyosin, the background, and the bulk density or lubrication effects. However, past studies concluded no single tenderness component can be used to predict beef tenderness for all cuts. Therefore, this study's objective was to better understand the relationships of various biochemical tenderness contributing components to the overall tenderness perception of 8 different beef muscles. Materials and Methods: Gluteus Medius (GM), Longissimus thoracis (LT), Pectoralis profundus (PP). Rectus abdominus (RA), Rectus femoris (RF), Semitendinosus (ST), Supraspinatus (SS), and Triceps brachii (TB) were collected from 10 UDSA high choice beef carcasses and assigned to a 2 or 21 d aging period (n = 160). Troponin-T (TNT) degradation, collagen content, pyridinoline (PYD) mature collagen crosslink density, intramuscular lipid content, pH, and trained panel analysis were measured. A Pearson correlation analysis was conducted to determine the relationship between each tenderness contributor measured in this study to the overall tenderness evaluated by the trained panelist. Results: All cuts increased in TNT degradation from 2 to 21 d postmortem. However, GM and RA displayed the least amount, while PP and TB showed the most TNT degradation compared to the rest of cuts after 21 d of aging (P < 0.05). The RF, SS and PP had the greatest collagen content followed by ST, TB, and GM, with LT and RA had the lowest collagen content (P < 0.01). For PYD density, PP and SS had the greatest densities followed by RA, ST, GM, TB, RF with LT displaying the lowest (P < 0.01). The RA and LT displayed the greatest lipid contents, followed by GM, RF, SS, and PP, with ST having the lowest lipid content (P < 0.01). The pH remained constant from 2 to 21 d of postmortem storage for all retail cuts except for LT and GM, which decreased after 21 d of aging (P < 0.01). The RA and SS displayed the greatest

pH values followed by RF, TB, PP, ST, and GM with LT displaying the lowest (P < 0.01). For overall tenderness evaluated by trained panelists, LT received the highest rating followed by TB, RA, RF, GM, ST, and SS with PP receiving the lowest overall tenderness rating (P < 0.01). The correlation analysis showed that the 8 cuts may have different profiles of tenderness contributors. For instance, overall tenderness for TB, ST, and RA may be driven by TNT degradation (r = 0.55, 0.45 respectively; P < 0.05). On the other hand, overall tenderness for PP may be driven by collagen content (r = 0.48; P < 0.05), and overall tenderness for GM was correlated with lipid content and pH (r = 0.51 and -0.74 respectively; P < 0.05). When all retail cuts were combined and analyzed holistically, all of the biochemical measurements conducted (except for pH) in this study played a small but important role as an overall tenderness predictor (P < 0.01). **Conclusion:** The results showed that muscle anatomical locations and physiological functions may explain the majority of the biochemical/tenderness differences found in this study, and 1 to 2 of the major biochemical factors may explain the overall tenderness for each muscle. However, the level of contribution to overall tenderness from each biochemical

factor varies greatly from one muscle to another.

Keywords: beef quality, Collagen, proteolysis, tenderness, trained sensory panel



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#### COMPARISON OF FRESH BELLY AND BACON CHARACTERISTICS BETWEEN COMMERCIAL DUROC SIRED AND HERITAGE BREED LARGE BLACK PIGS

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**Objectives:** Consumer demands for healthier products shaped the industry to produce leaner pork. Large Black Pigs are a pasture raised heritage breed that is a part of an emerging niche market and has not undergone major genetic selection for percent lean. Therefore, limited data is available comparing Large Black belly and bacon quality traits to commercial breeds. The objectives of this study were to examine differences in fresh belly and bacon characteristics between commercial Duroc-sired and Large Black genetic lines fed high forage or commercial diets. **Materials and Methods:** Fifty pigs were used in the study, Duroc sired (DS, n=25 pigs) and Large Black sired (LB, n=25 pigs). Pigs were assigned to one of two dietary treatments: Fiber (Fib) or Control (Con), using a 2 x 2 factorial design of breed and diet. Dietary treatments were fed throughout the grow-finish period (101 or 140 d) in six phases. Con diet was corn-soybean meal-DDGS based and Fib diet used increasing amounts of wheat middlings (1-10%) and dehydrated alfalfa meal (7.5-20%) replacing corn and soybean meal in the Con diet, from phase 1-6. Pigs were harvested at a

common age, but BW varied between genetics (DS 125 ± 2.23 kg, LB 99 ± 2.28 kg; *P*<0.001). Fresh bellies (IMPS 408) were obtained from each carcass and weighed (fresh weight) and measured for firmness by suspending the bellies over a PVC pipe (d=8.89cm) with skin side down; greater distance between the blade and flank ends of a belly indicated increased firmness. Fresh bellies were injected to 110% fresh weight with a manual compressed air stitch pump, thermally processed to 62°C, cooled to 1°C internal temperature, and weighed (cooked weight). Processing yield was calculated as a percentage: (cooked wt / fresh wt) x 100. A 0.64 cm bacon slice was removed at 25, 50 and 75% distance from the blade end of the cooked bellies for visual image analysis. Images of the slices were analyzed with Adobe Photoshop for bacon total slice length (SL; cm), total slice area (SA; cm<sup>2</sup>) and lean area (LA; %). Data were analyzed with breed and diet as fixed effects using RStudio (1.2.1335) with least square means separated at (*P*<0.05). **Results:** Results showed differences in firmness due to a breed x diet interaction (*P*=0.0527); LB Con were the firmest bellies (*P*<0.01), with LB Fib intermediate in firmness (*P*=0.0325), and DS Con and DS Fib were not different (*P*=0.5577). DS bellies had greater (*P*<0.01) processing yield than LB bellies. A tendency for a breed effect was observed in SL with DS slices longer than LB slices (*P*=0.065). Diet was significant for SA as slices from Con bellies were larger than Fib slices (*P*<0.01). Breed was significant for LA, as DS slices had greater LA than LB slices (*P*<0.01). No breed x diet interaction was

found in slice images.

**Conclusion:** This experiment found variations in fresh belly and bacon characteristics between DS and LB genetic lines and their diets. LB bellies had decreased LA, indicating a reduced proportion of lean throughout the belly. This supports the decreased processing yield, as LB bellies would have less protein to absorb brine, yet improved firmness with more fat. While LB pork may have niche market value, the integration of this breed into commercial bacon processing has limitations in composition that need to be further evaluated to improve the product desirability.

Keywords: Bacon, Duroc Sired Pigs, Heritage Breed, Large Black Pigs, Pork Quality



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### EFFECTS OF FRESH BEEF TUMBLING AT DIFFERENT POSTMORTEM TIMES ON QUALITY AND PROTEOLYTIC ATTRIBUTES OF M. LONGISSIMUS LUMBORUM

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**Objectives:** Supplying consumers with consistently tender beef products remains a significant challenge in the industry. While tumbling has been extensively practiced for processed meat with brine addition, tumbling alone is not considered as a means to improve beef tenderness. It has, however, recently been demonstrated that tumbling without incorporation of a brine solution can be successful in improving the instrumental tenderness and consumer liking of tenderness of beef loin (*M. longissimus lumborum*). At present, though, the time at which the tumbling treatment should be applied to be most effective at optimizing quality attributes remains unclear. The objective of this study was to determine the most effective postmortem time point to apply tumbling to enhance tenderness and proteolytic features of beef loin.

**Materials and Methods:** Beef loin muscles (n=12; IMPS 180, USDA low Choice) were obtained at 1d postmortem from carcasses, then sectioned and assigned to tumbling (T) time point treatments [non-tumbled (NT), 1d-T, 6d-T, and 11d-T] with equal distribution among muscle positions. Sections were tumbled for 90 min (non-vacuum, 8.5 rpm) at the assigned tumbling time, then steaks (2 cm thick) were made. Steaks were randomly assigned to either no aging or further aged to a common postmortem time of 16d. Meat quality measurements included Warner-Bratzler shear force (WBSF), instrumental color, water-holding capacity (WHC), and pH. Western blot analyses of desmin and troponin T degradation, calpain-1 autolysis, and myofibril fragmentation index (MFI) were performed to assess proteolytic activity. All treatment levels were analyzed using one-way ANOVA and Tukey pairwise comparison test of SAS 9.4. Statistical significance level was set at  $P \le 0.05$ .

**Results:** Tumbling beef loins at 1d and 6d postmortem resulted in lower WBSF values (P<0.05) compared to the NT controls. Tumbling early postmortem (1d) with no further aging had similar WBSF values compared to NT loins at 16d (P>0.05). Tumbling at 6d and 11d with further aging resulted in the lowest WBSF values (P<0.05), though no differences were found between different tumbling treatments (P>0.05). In general, fresh beef tumbling had minimal impacts on pH, color attributes, and WHC, regardless of the time point (P>0.05). Degradation of troponin T and desmin, and calpain-1 autolysis, were not affected by tumbling treatments (P>0.05), though proteolysis was increased with aging (P<0.05). Although not significant, numerical increases in MFI were found in samples assigned to tumbling at 1d and 6d postmortem. Significant increases in MFI, however, were found in loins assigned to tumbling at 11d postmortem compared to NT counterparts.

**Conclusion:** The results of the current study confirm that fresh beef tumbling alone without brine addition can improve beef instrumental tenderness and could considerably shorten postmortem aging times. Further, the current study found that the positive impacts of beef tumbling may be further maximized through early postmortem application, which would likely be attributed to physical disruption to muscle structure coupled with additional enzymatic degradation. Future studies should evaluate the postmortem time of fresh beef tumbling on sensory attributes of beef muscles to identify the optimal process to improve eating quality and product consistency.

Keywords: beef quality, meat tumbling, postmortem proteolysis, tenderness



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#### **ANAEROBIC DRY AGING OF BEEF**

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**Objectives:** Dry aging has recently gained popularity among high end restaurants and smaller meat packing plants looking to add value to their beef. Dry aging is known for the development of unique flavors via concentration of flavor compounds through dehydration and the creation of new flavors through oxidation and endogenous enzymatic processes. However, prolonged exposure of meat to cooler conditions, which is common practice in dry aging processes, can lead to off-odors and flavors. The objective of this research project was to determine the effects of removing oxygen from the beef dry aging system. The hypothesis was that oxygen removal would result in a reduction/inhibition of oxidative products and a superior dry aged beef flavor.

Materials and Methods: Boneless upper 2/3 Choice beef strip loins (n=18) were randomly assigned three aging treatments: wet aging, traditional (aerobic) dry aging, and anaerobic dry aging. All treatments were aged for 41 days at  $2 \pm 1^{\circ}$ C. The dry aged treatments were held at 50% relative humidity (RH) with a fan speed of 2,200 revolutions per minute (RPM). Anaerobic dry aged samples were aged in dry aging chambers housed within an oxygen impermeable film bubble. Complete anaerobic conditions were not achieved with the system in that the oxygen concentration at the surface of the meat remained 1-5%. To achieve anaerobic conditions, anaerobic chambers were flushed with an 80% nitrogen (N) 20% carbon dioxide (CO<sub>2</sub>) gas mixture at the beginning of aging and any time the oxygen concentration approached 4%. Food grade oxygen scavengers were also utilized to remove residual oxygen after gas flushing. After the aging process was completed, dry aged samples were weighed before and after trimming to determine yield. **Results:** There was a significant difference in yield between the wet aged (95% yield) and the two dry aged treatments (p < 0.05), but there was no significant difference between the anaerobic (55% yield) and aerobic (54% yield) dry aging treatments. Trimmed loins were then cut into steaks, packaged, and frozen (-80°C or -20°C depending on analysis) for further analysis. Lipid oxidation was measured using thiobarbituric acid reactive substances (TBARS). There was no significant difference found in TBARS between the wet aged (1.18 mgs Malonaldehyde/kg of tissue) and anaerobic dry aged (1.27 mgs M/kg) samples (p < 0.05), but there was a significant difference between the aerobic dry aged (2.46 mgs M/kg) samples and the other two treatments (p < 0.05). A paired preference test was conducted to determine consumer flavor preference between anaerobic and traditionally (aerobic) dry aged steaks. Sensory steaks were cooked to medium well (70°C) and then cut to a sample size of 2 cm x 1 cm x 2.54 cm. There were no significant differences found among treatments (p < 0.05). Finally, a trained sensory analysis was conducted. Anaerobic dry aged samples tended to have more desirable flavor notes and aerobic dry aged beef tended to have more undesirable flavor notes. **Conclusion:** These results suggest that although anaerobic dry aging significantly reduces lipid oxidation, it is unclear if this difference is detectable to consumers. More research is required on the pathways of flavor development of dry aged beef.

Keywords: Dry Aging, flavor, oxidation



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### COMPARISON OF BONE-IN AND BONELESS AGING OVER EXTENDING AGING PERIODS ON CONSUMER RATINGS OF BEEF LOIN STEAKS

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**Objectives:** The objective of this study was to evaluate the influence of bone-in aging over extended periods on consumer ratings of beef steaks.

**Materials and Methods:** Paired short loins were selected from beef carcasses (n = 20) from the upper 2/3rds of USDA Choice (Modest<sup>00</sup>-Moderate<sup>100</sup> marbling scores). Following collection, the right-side short loins were deboned into strip loins, then each sub primal was portioned into three equal sections. Sections were vacuum packaged and randomly assigned to one of three aging periods: 21, 42, or 63 d. After each respective aging period, sections were fabricated into 2.54 cm steaks, then steaks were vacuum packaged and frozen at -20°C until further analysis. Prior to consumer panel analysis, steaks were thawed at 1-4°C for 24 h prior to panels. On bone-in steaks, the bone was retained during cooking. Steaks were cooked on an open face clamshell grill until internal temperature of 71°C was reached. Immediately prior to serving, steaks were cut into steak thickness × 1 × 1 cm cubes, then 2 cubes were served to each panelist. Each panelist was served one sample of each treatment in a randomly assigned order and rated each sample for flavor liking, tenderness, juiciness, and overall liking using a digital survey on an electronic tablet. Panelists were also asked to rate each trait as acceptable or unacceptable and assigned a quality level to each sample. Data was analyzed as a split plot design, with sub primal serving as the whole plot and section serving as the subplot. Aging type, period, and the interaction served as the fixed effects, and panel was incorporated into the model as a random effect. Acceptability data was analyzed as a binomial distribution.

**Results:** No interaction for aging type × period was observed for any traits evaluated ( $P \ge 0.146$ ). Additionally, no differences were observed between aging periods for any traits evaluated ( $P \ge 0.078$ ). However, consumers determined that bone-in steaks were juicier (P < 0.05) and preferred bone-in steaks overall (P < 0.05) compared to boneless steaks. No differences were observed between boneless and bone-in steaks for tenderness and flavor liking ( $P \ge 0.093$ ). Additionally, no differences were observed between aging type for acceptability of any trait ( $P \ge 0.402$ ). However, a greater percentage of consumers rated bone-in steaks as premium quality P < 0.05) compared to boneless steaks. **Conclusion:** These results indicate that, despite anecdotal evidence suggesting a consumer preference for bone-in steaks, they perform similarly to boneless steaks for tenderness and flavor. However, bone-in steaks are juicier and liked more overall by consumers, which indicates a more premium eating experience for the consumers.

Keywords: aging, Bone-In, consumers, extended aging, palatability



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#### PROFILING FLORIDA RAISED BEEF FOR QUALITY AND TENDERNESS ATTRIBUTES

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**Objectives:** Improvements in genetics, nutrition, and management practices have positively affected the palatability of beef from Florida-raised cattle bringing products nearer to the levels associated with beef from the rest of United States. However, industry perceptions of meat quality attributes of Florida cattle have changed relatively little over time, negatively impacting valuation of cattle from the Southeastern region. The objective of this study is to investigate the claim that Florida-raised beef is equal in quality and tenderness to beef produced elsewhere in the United States.

#### **Materials and Methods:**

Quality and tenderness attributes of Florida raised beef versus controls					
	Florida Ranches (n = 168)	Steak Cutter (n = 50)	Retail Outlet (n = 22)	P value	
Marbling Score	378	435	380		
Slice Shear Force, kg	22.7 ± 0.5 <sup>a</sup>	17.5 ± 0.6 <sup>b</sup>	16.5 ± 0.8 <sup>b</sup>	< 0.001	
Sensory Tenderness	5.2 ± 0.1	5.9 ± 0.2	6.0 ± 0.2	0.12	
Sensory Juiciness	5.2 ± 0.1	5.1 ± 0.1	4.8 ± 0.2	0.20	
Sensory Beef Flavor	5.5 ± 0.1	5.5 ±0.1	5.3 ± 0.1	0.25	
Sensory Connective Tissue	6.2 ± 0.1	6.7 ± 0.2	7.0 ± 0.2	0.09	
Sensory Off Flavor	5.7 ± 0.1 <sup>ab</sup>	5.8 ± 0.1 <sup>a</sup>	5.4 ± 0.1 <sup>b</sup>	0.03	

Steers (n = 168) from 9 ranches in Florida were finished at a commercial feedyard in North Central Florida. Steers were harvested on three dates after 162, 197 and 211 d of feeding when it was visually determined that they had 1.2 cm of backfat. All cattle were harvested at a local packing plant and carcasses were chilled for 48 h before samples were collected. Approximately 72 h postmortem 2, 2.5 cm thick steaks were cut from the strip loin prior to carcass fabrication, vacuum packaged and allowed to age in a cooler for 14 d prior to being frozen. Control steaks for comparison were procured from a custom steak cutter (USDA low Choice). In addition, control strip steaks were procured from a local retail outlet (USDA Select). The strip steaks obtained from the steak cutter (n=50) and the retail outlet (n=22) were purchased in similar percentages of Choice and Select to the USDA grade on the Florida cattle. Steaks were vacuum packed and aged for 15 d before freezing. Slice shear force data was obtained for all steaks over a 4d period. All steaks were thawed for 24 h in a cooler and subsequently cooked to 71°C. Three slice samples were taken from each steak using a 90-degree slice box and sheared on an Instron machine. Trained sensory panel analysis was conducted on all steaks over 27 d. All data were analyzed using SAS with steak origin as treatment and degree of doneness as a covariant. **Results:** Steaks from Florida cattle had higher slice shear force values than steak cutter or retail outlet steaks (p < 0.001). Trained sensory panelists did not detect significant differences in Florida cattle compared to steak cutter or retail outlet steaks for tenderness, beef flavor, connective tissue, juiciness, or off flavor.

Conclusion: Differences in slice shear force values among treatment did not translate to differences in sensory panel values. Majority of the samples tested in this study represent the 100 degrees of marbling between Slight 50 and Small 50. This small range may have contributed to less consistent sensory panel evaluations.

Keywords: Beef quality, Bos indicus, Tenderness



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#### PREDICTING CARCASS COMPONENT YIELDS FOR TURKEYS USING SHACKLE IMAGES

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**Objectives:** Yields deriving from the different carcass cut-up parts of a turkey are considered to be major factors in poultry breeding. The yield measurements from each part, including those of high interest for meat production such as breast, thighs, and drums, are known to have moderate to high heritabilities and therefore have played an essential role in genetic selection. However, manually collecting yield measurements has become problematic, as labor costs can be extensive especially considering the number of phenotypes that need to be collected in order for it to be relevant for genetic selection. The objective of the current study was to use the data collected from image analysis software of still shackle images, in combination with previously recorded weights from different turkey carcass components to create a predictive model for future component yield estimations.

**Materials and Methods:** In this study, we experimented using a commercial image analysis system, developed primarily for broiler chicken, that is better able to assess this issue. Note: this particular system has never been used to estimate turkeys' meat yield. The system was originally developed to enable the assessment of the carcass as a whole and then determine its grade for further processing. It uses advanced imaging software to evaluate carcass shape, color, texture, as well as provides distinct coordinates. The data collected allows for the dimensions of the broiler carcass to be calculated. Images for approximately 10,000 toms originating from three different pedigree lines, were captured after the carcasses were de-feathered and eviscerated prior to chilling. Commercial processing procedures were met at each stage as conducted in a Canadian government inspected processing plant. The weight of both *pectoralis major* and *minor* were recorded after chilling and meat maturation (24hrs). Additionally, one third of the toms had weights recorded for thighs, drums, wings, scapula, breast skin, and rack.

**Results:** Moving forward, several predictive models are currently being created and tested using multiple linear regression, regression trees, and neural networks.

**Conclusion:** The results will provide the industry an advanced, more effective method of turkey carcass yield estimations for both the whole carcass and the parts of interest. In addition, the dimensions of each bird possessed in the study will be saved and aid in future genetic selection within turkey pedigree lines.

Keywords: image analysis, yield



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### A COMPARISON OF MEAT QUALITY AND SENSORY ATTRIBUTES IN FRESH AND FROZEN AMERICAN LAMB USING TWO DIFFERENT MUSCLES

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**Objectives:** The objective of this study was to evaluate differences in meat quality and sensory attributes of fresh and frozen lamb using the *longissimus lumborum* (LL) and *semimembranosus* (SM) muscles.

**Materials and Methods:** Dorset wether lambs raised at North Dakota State University (NDSU)(n=12) were harvested at the NDSU Meats Laboratory. After a 24 h chill, loin and leg subprimals were collected from each carcass. Subprimals were split in half and each side was assigned to either fresh (FRSH) or frozen (FRZN) treatment. Each half was weighed before being vacuum sealed. Subprimals assigned to FRSH treatment were stored in a cooler at 3° C for 14 days while subprimals assigned to FRZN treatment were stored in a freezer for 13 days + 1 day of thawing at 3° C. Before fabrication, subprimals were removed from bags and reweighed for primal weight loss. After weighing, loin subprimals were fabricated starting at the cranial end, while the SM was removed from the leg subprimal and fabrication began at the distal end. For both muscles, an ~1.27 cm chop was removed for drip loss analysis, an ~2.54 cm chop was fabricated, vacuum sealed and stored at 3° C for Warner-Bratzler shear force (WBSF) and cook loss evaluation, and remaining chop samples were used for sensory evaluation. Drip loss was determined over 24 hrs from suspended 25 g samples. WBSF and cook loss analysis was conducted in accordance with AMSA guidelines. Sensory evaluation was conducted 24 h after sample fabrication. Consumer panelists (n=84) were given paired samples of LL and SM and were asked to evaluate overall likeness, flavor like, tenderness like, and juiciness like on a continuous line scale. Data were analyzed using the PROC Mixed procedure SAS Studio<sup>°</sup> (SAS Institute, Cary, NC) with means being separated with the PDIFF option and were considered significant when  $P \le 0.05$ .

**Results:** No differences were observed between treatments for primal weight loss, cook loss, or Warner-Bratzler shear force in LL-FRSH, LL-FRZN, SM-FRSH or SM-FRZN ( $P \ge 0.10$ ). However, LL-FRSH and SM-FRSH samples experienced significantly less drip loss compared to LL-FRZN and SM-FRZN samples (P < 0.0001, P = 0.0003, respectively). LL-FRSH sensory samples had significantly higher overall like, tenderness, and juiciness scores compared to LL-FRZN samples (P = 0.01, P = 0.02, P = 0.03, respectively). However, no differences in flavor scores were observed in LL-FRSH samples compared to LL-FRZN samples. Additionally, no differences in overall like, flavor, tenderness, or juiciness scores were observed in SM-FRSH samples compared to SM-FRZN samples ( $P \ge 0.77$ ).

**Conclusion:** Our results indicate no influence on meat quality and sensory attributes of SM to lamb when frozen. Industry application of frozen storage may deem beneficial for supply of legs. However, we did identify negative influence on sensory attributes to lamb LL by freezing, specifically related to water holding capacity and juiciness. The differences in overall consumer likeness can be attributed to the perceived juiciness and tenderness, while flavor profiles of fresh vs. frozen lamb were indistinguishable. Therefore, maintaining fresh lamb loin chops in retail and foodservice offer the greatest opportunity for consumer satisfaction.

Keywords: fresh and frozen, lamb quality, sensory attributes



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EFFECTS OF DIFFERING CARBON DIOXIDE LEVELS ON MEAT COLOR IN MODIFIED ATMOSPHERE PACKAGING

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Objectives: The most important factor influencing consumers' purchasing decision of meat is color. Consumers associate a bright cherry-red color in beef with wholesomeness and freshness. Modified atmosphere packaging (MAP) allows removal or replace the gaseous environment surrounding a product before sealing the package. Tri-gas (Nitrogen 69%, carbon monoxide 0.4%, carbon dioxide variable) carbon monoxide MAP is effective in stabilizing color as the carbon monoxide (CO) binds to the myoglobin and forms carboxymyoglobin. During COVID-19 pandemic, carbon dioxide (CO<sub>2</sub>) was in short-supply and most gas flushes in mother bags or MAP use 30% CO<sub>2</sub>. Therefore, the objective of this study was to assess the effect of different CO<sub>2</sub> concentration gas flushes in master packages on the display life of fresh beef. Materials and Methods: Twenty-five USDA Low Choice ribeye rolls (IMPS 112A) were collected from a commercial processing facility. Each sub-primal was wet aged for 12 d. After aging, each sub-primal was opened and surface sprayed with a proprietary antimicrobial solution. Ribeye rolls were sliced 2.54 cm thick and placed onto foam trays in adjacent pairs (N = 108 trays) with an absorbent pad and over-wrapped with perforated polyolefin film. Trays were randomly assigned to either a 10%, 20% or 30% CO<sub>2</sub> concentration master bag (n = 27 master bags;  $n = 9/CO_2$  concentration). Three additional master bags per CO<sub>2</sub> concentration were packaged and assigned to headspace analysis. Oxygen, carbon dioxide, and carbon monoxide were evaluated at 0, 6, 24, 30, and 48 h. Three master bags, containing four trays, for each  $CO_2$  concentration (10%, 20%, and 30%) were randomly selected for opening after 10, 15, 18 d of dark storage. Following respective storage, the trays were placed in a retail display case for 5 d color evaluation. Headspace analysis was conducted before each master bag was opened, and objective color measurements were collected on d 0 - 5 of retail display. Muscle color, surface discoloration, and overall acceptability were analyzed daily by a trained panel (n = 6). **Results:** Residual oxygen content within master bags containing 20 and 30% CO<sub>2</sub> decreased to 0% in 48h. However, the master bags flushed with 10% CO<sub>2</sub> contained more O<sub>2</sub> over the entire 48 h period and never decreased below the goal of less than 0.15% residual oxygen. As a result, steaks from master packages flushed with 10% CO<sub>2</sub> darkened and discolored more (P < 0.05) rapidly than steaks from either the 20 or 30% CO<sub>2</sub> concentrations. Additionally, steaks from master bags flushed with the 10% CO<sub>2</sub> concentration were the only steaks in display to reach unacceptable levels. Steaks from master bags flushed with 20 or 30% CO<sub>2</sub> concentration performed comparably (P > 0.05). Conclusion: The 20% CO<sub>2</sub> concentration master bag flush outperformed the 10% CO<sub>2</sub> concentration in all aspects of the study, as well as surpassed or equaled the 30% CO<sub>2</sub> concentration. Thus, it is recommended 20% CO<sub>2</sub> concentration gas

**Keywords**: Carbon dioxide, Meat color, Modified atmosphere packaging

flush of master bags could be utilized by the industry to reduce CO<sub>2</sub> use.



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#### DESCRIPTIVE ASSESSMENT OF DRY-AGED BEEF FROM COMMERCIAL AGING LOCATIONS ACROSS THE UNITED STATES

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**Objectives:** The effect of dry-aging on beef flavor and unique flavor attributes is equivocal in the literature. The objective of this study was to determine the influence of commercial dry-aging on the intensity of beef flavor attributes through a trained descriptive sensory panel.

**Materials and Methods:** Bone-in beef strip loins (NAMI # 175) were purchased from a commercial beef plant (N = 66). Samples were obtained from the same lot and date of production. Strip loins were fabricated from carcasses that were identified by United States Department of Agriculture (USDA) – Agricultural Marketing Service grading personnel to meet Certified Angus Beef<sup>®</sup> (USDA Schedule G1) brand specifications and were obtained from the left side of each carcass so as to not have duplicate strip loin samples from any single carcass. Six strip loins were shipped to each commercial aging location, aged for 45-days, and returned to the University of Idaho. An additional six strip loins were wet-aged for 45 days as a negative control. Following aging, strip loins were fabricated end-to-end into 2.54 cm thick steaks, vacuum packaged and frozen (-28°C) until further analysis. Trained sensory analysis was performed using the methods described in the American Meat Science Association Sensory Guidelines. In addition, training was developed to calibrate panelists for flavors commonly associated with dry-aged beef: earthy, yeasty, nutty, cheesy, sour, bitter, sweet, and flavor intensity. Data was analyzed using the mixed model and correlation procedures of Statistical Analysis Software. Significance was determined at P < 0.05.

**Results:** Dry-aged beef flavor intensity was different among aging locations (P < 0.01). Following additional analysis to separate aging locations among mild and bold flavors further analysis was conducted within flavor intensity classifications. Among the mild intensity flavor locations there were differences in cheese flavor notes (P < 0.01). Moreover, among mild flavor intensity locations there was a positive correlation (r = 0.45; P < 0.01) between earthy and bitter flavors and a negative correlation (r = -0.59; P < 0.01) among yeasty and nutty flavors. Additionally, among the bold intensity locations there were differences flavor notes (P < 0.01). Among the bold flavor intensity locations between cheesy and bitter flavors (r = -0.43; P < 0.01), sour and nutty flavors (r = -0.52; P < 0.01), and nutty and bitter flavors (r = -0.59; P < 0.01).

**Conclusion:** The current research indicates that dry-aging imparts distinguishable flavor characteristics on the final dry-aged product where distinct differences in commercial dry aging locations can be observed.

Keywords: aging parameters, beef, dry-aging, flavor, sensory attributes



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### FREE AMINO ACID CONTENT IN LONGISSIMUS LUMBORUM AND SEMIMEMBRANOSUS MUSCLES FROM ELECTRICALLY STIMULATED BEEF CARCASSES

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**Objectives:** Free amino acids in postmortem beef muscles contribute to the development of cooked beef flavor. Both electrical stimulation (ES) and aging increase postmortem proteolysis, thereby accelerating the accumulation of free amino acids in postmortem tissues. This study examined the combined effects of electrical stimulation on free amino acid content in beef *longissimus lumborum* (LL) and *semimembranosus* (SM) muscles aged for 1, 4, and 14 d. **Materials and Methods:** Twenty-three crossbred beef carcasses were randomized to receive either extra-low voltage (20 V for 20 s) electrical stimulation (ES; n = 11) or non-stimulation (NS; n = 12) at exsanguination. Again at 1 h postmortem, two sides of each carcass were randomized to receive either ES or NS, resulting in two early ES treatments (ES-ES and ES-NS) and two early NS treatments (NS-NS and NS-ES). Samples were removed from the LL and SM for free amino acid analysis on d 1, 4, and 14 postmortem. Free amino acids in the filtrate were combined with norvaline as internal standard, derivatized with propyl chloroformate, and determined by gas chromatography - mass spectrometry. Data were analyzed as a split-plot design in time in a generalized linear mixed model with treatment, day, and their interaction as fixed effects and carcass side within treatment as a random effect. Actual probability values were reported.

**Results:** Thirty-one amino acids were quantified in beef LL and SM muscles. The predominant amino acids were alanine,  $\beta$ -aminoisobutyric acid, and glycine (553 to 1901 nmol/g); whereas the least predominant amino acids were cystine,  $\alpha$ aminobutyric acid, and thiaproline (8 to 14 nmol/g). The treatment effect was found only for ornithine in SM (P = 0.030), with early ES treatments increasing ornithine by 15 to 19 nmol/g ( $P \le 0.045$ ). Two-way treatment × day interactions were found for glutamic acid and asparagine in SM (P < 0.001). Glutamic acid remained similar among treatments on d 1 and 4; however, it was increased by 213 to 247 nmol/g on d 14 by early ES compared with early NS (P < 0.001). Asparagine followed a similar pattern with an increase of 20 to 28 nmol/g by early ES on d 14 ( $P \le 0.048$ ) compared with early NS. Other free amino acids in SM, including the most predominant ones, were influenced only by day with d-14 content being the greatest ( $P \le 0.023$ ). In the LL, only day effect was found for 18 amino acids ( $P \le 0.038$ ), including those related to desirable flavors such as glutamic acid (umami) and glycine (sweetness).

**Conclusion:** Early electrical stimulation influenced the free amino acid content in the SM muscle more than that in the LL muscle. However, aging increased the content of most amino acids across treatments and muscles. These findings suggest that both electrical stimulation and aging can be used to alter the content of water-soluble flavor compounds in beef, which, in turn, influence beef flavor and consumer acceptance.

Keywords: beef flavor, water-soluble, amino acids



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### IMPACT ON ANTIMICROBIAL INTERVENTIONS ON SPOILAGE BACTERIA DURING EXTENDED STORAGE OF RAW, VACUUM PACKAGED BEEF FROM TWO FACILITIES

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**Objectives:** Fresh, chilled, vacuum packaged beef accounts for an estimated \$4 billion of the annual US beef export market. Retarding microbiological spoilage is a crucial step in improving the shelf life and adding value to the sector. Organic acid antimicrobials are one method used to address spoilage organisms unique to an individual processing facility. The objective of this study was to determine the effect of processing plant and organic acid treatment on the microbial profile of raw beef products.

**Materials and Methods:** Beef chuck rolls (IMPS 116A, N=24) were obtained from two different processing facilities on two different days of production. On day 7 postmortem, six chuck rolls from a single plant and processing day were cut in half and assigned to one of four treatments: 4.5% lactic acid, 2.5% Beefxide, 380 ppm peroxyacetic acid, or a notreatment control. Each half-chuck roll was cut into at least 6, ca. 1 kg pieces, submerged into the assigned organic acid solution for 15 seconds, allowed to drip for two minutes, and individually vacuum sealed. Samples were stored at 2.7 °C for 112 days. On day 0 (day of organic acid treatment) and every 28 days thereafter, aerobic, anaerobic, psychrotrophic, lactic acid bacteria, and *Pseudomonas* plate counts were measured on a single piece. A 100 g sample was cut from the surface of each piece and stomached with 100 mL of buffered peptone water. Homogenate was plated in duplicate onto brain heart infusion agar for aerobic, anaerobic, and psychrotrophic plate counts; deMan Rogosa Sharpe agar for lactic acid bacteria; and cephaloridine fucidin cetrimide agar for *Pseudomonas*. Aerobic, anaerobic, lactic acid, and *Pseudomonas* plates were incubated at 37 °C for 48 hours. Psychrotrophic plates were incubated at 4 °C for 10 days. The experiment was conducted in two independent replications with one day of production from each facility included in a single replication. Samples stored for 56 days from the second location were not evaluated due to a laboratory closure that prevented sampling. Data were analyzed as an incomplete block design with 2 locations, 4 treatments, and 5 sampling days in SAS 9.4.

**Results:** Bacterial counts increased during storage across all microbial populations (P < 0.01). The impact of treatment was only significant for the *Pseudomonas* counts (P = 0.04) where lactic acid (P = 0.01) and Beefxide (P = 0.03) treatments had lower *Pseudomonas* concentrations than the control group. *Pseudomonas* (P < 0.01) and psychrotrophic (P < 0.01) counts were lower in samples from location 1. Anaerobic (P = 0.04) and lactic acid bacteria (P < 0.01) counts were lower in samples from location 2.

**Conclusion:** Bacterial counts increased during storage, and differences between treatments and locations were minimal. Although significant differences existed between locations, these differences were less than 1 log and may not be biologically relevant. No treatment impacted the concentrations of lactic acid bacteria suggesting that organic acids have little to no effect on the growth of lactic acid bacteria in raw, vacuum packaged meat. Lactic acid and Beefxide were able to retard the growth of *Pseudomonas* compared to untreated control samples. Collectively, this suggests that the use of organic acids for shelf-life extension should target specific groups of spoilage bacteria.

Keywords: beef, Export, Microbial Spoilage, Organic acids



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#### VALORIZATION OF GRAPE POMACE FOR A FUNCTIONAL ADDITIVE IN HAMBURGER PATTIES

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**Objectives:** Conversion of food wastes into functional products can create a win-win situation with food waste reduction, value-addition, and additional income to product growers. The purpose of this research was to assess antioxidant activity and phenolic content of seed and seedless pomaces as well as evaluate product quality and sensory attributes of hamburger patties made with grape pomace.

Materials and Methods: Two types of white-wine grapes (Malvasia and Sauvignon Blanc) were obtained from a commercial winery vineyard. The impurities (vine, branches, and leaves) of the grapes were manually removed before producing grape pomace by squeezing using a pressor. The pomace was then separated into seed and seedless pomace. The pH and instrumental color of pomace were measured. Following pulverization of dried seeds and freeze-dried seedless pomace, using liquid nitrogen, both seed and seedless pomace were subjected to an ABTS and Folin-Cioulteau assays to measure the relative ability of antioxidants and total phenols, respectively. Hamburger patties were prepared with seeded pomace using 0% control, 5% Malvasia, and 5% Sauvignon Blanc which were vacuum packed and stored at -21 °C until further use. Frozen patties were thawed and cooked to an internal temperature of ~ 76.7 °C using a conventional oven prior to wrapping and chilling. Cooked patties were measured for instrumental color and cooking loss Consumer sensory was conducted using a 50 sensory panel for appearance, aroma. overall, taste, juiciness, sweetness, texture, firmness, after taste, and willingness to buy. Data in three replications were statistically analyzed using the GLM procedure of SAS as a completely randomized design. If a significance was determined (P < 0.05) in the model, dependent variable means were separated using the least means significant difference procedure of SAS. **Results:** Results of ABTS assay indicated that Sauvignon Blanc seeds possessed higher radical scavenging activity than Malvasia (P < 0.05). A similar trend was observed in the seedless pomace although the activity was relatively lower than that of the seeds (P < 0.05). Malvasia seeds showed higher phenolic content than Sauvignon Blanc, however, no difference of phenolic content was found between the two pomaces (P < 0.05). Sauvignon Blanc pomace displayed more yellowness (\*b) than Malvasia pomace, with no differences in brightness (L\*) and redness (a\*). No pH difference was found between the two pomaces (P < 0.05). Upon cooking, control patty showed more lightness (L\*), redness (a\*) and yellowness (b\*) than those of pomace patties (P < 0.05). No significant difference of cooking yield was found, regardless of treatment. No significant difference of sensory attributes was observed for hamburger patties regardless of treatment (P < 0.05).

**Conclusion:** Based on these results, addition of white wine grape pomaces may increase antioxidant activity, phenol level and increase health benefits of hamburger patties. The prospect of reducing the waste issue of grape pomace with the potential of additional income to grape processors is a step toward a more sustainable food chain. Further research is required to evaluate the benefits of grape pomaces from other grapes such as red wine grape and juice type grape in various processed meat products such as sausage, meat ball, meat loaf, ground meat etc.

Keywords: antioxidants, grape pomace, hamburger patty, phenol, sensory



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#### SHELF LIFE EXTENSION OF RAW GROUND TURKEY USING VINEGAR AND NATURAL FLAVORS

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**Objectives:** This study quantified the ability of Vinegar and Natural Flavor (V/NF) and Natural Flavor (NF) to enhance the oxidative stability of fresh ground turkey, while minimizing quality impacts.

**Materials and Methods:** Fresh, ground turkey was received at the Corbion meat processing facility, approximately 24 h post-mortem. The ground turkey was mixed in 40-pound batches for 3 minutes, following the addition of 1.05% V/NF or 0.95% NF. Following mixing, 200g samples were portioned into 3 mil vacuum bags, vacuum sealed, and stored at 3.3°C. Samples were stored in the dark, to simulate vacuum packaging in a light impermeable film. Samples were analyzed throughout the cold storage period (21 days) for instrumental color (HunterLab, L\*a\*b\*), oxidative rancidity (TBA Value), and sensory attributes (Descriptive Analysis). Instrumental color was collected on the same 8 samples throughout the duration (day 0, 2, 7, 9, 14, 16, 21) of the shelf-life study. Similarly, 200g samples were collected at selected dates (day 0, 7, 9, 14, 16, 20) throughout the shelf life, and stored at -80°C until TBA analysis was performed. Finally, sensory analysis was performed at day 1 and 14, using a trained sensory panel. Members were trained to specifically recognize oxidized, warmed over, or rancid off-flavors.

**Results:** The application of V/NF and NF at 1.05% and 0.95%, respectively, enhanced the oxidative stability of fresh ground turkey, while minimally impacting the fresh meat color and organoleptic properties of the cooked product. After 20 days of storage, the TBA value of the samples treated with V/NF (0.289 mg MDA/kg sample) and NF (0.250 mg MDA/kg sample), were below the detectable sensory threshold of 0.50 mg MDA/kg sample. In contrast, the TBA value of the control (1.42 mg MDA/kg sample) far surpassed the previously stated sensory threshold. Additionally, no significant differences in color (L\*a\*b\*) values were observed with respect to treatment throughout the storage period. Finally, no significant differences (P<0.05) were observed between the control samples and the samples treated with the V/NF and NF, 1-day post-production, when the samples were analyzed using a trained sensory panel.

8	Treatment			
Day	Control	1.05% V/NF	0.95% NF	
0	0.398	0.382	0.211	
7	0.320	0.164	0.179	
9	0.296	0.234	0.164	
14	0.515	0.211	0.094	
16	0.694	0.304	0.187	
20	1.420	0.289	0.250	

Table 1. TBA values (mg MDA/kg sample) for vacuum packaged, raw, ground turkey stored at 3.3°C.

**Conclusion:** Throughout a 20-day shelf life, the inclusion of V/NF and NF inhibited lipid oxidation. Therefore, the application of V/NF and NF may be used to extend the shelf life of fresh ground turkey, without negatively impacting the organoleptic properties of the fresh or cooked ground turkey.

Keywords: color stability, sensory attributes, Turkey



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#### GENETIC PARAMETER ESTIMATES FOR MEAT QUALITY TRAITS IN CANADIAN TURKEYS (MELEAGRIS GALLOPAVO) USING GIBBS SAMPLING

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**Objectives:** With an increasing number of poultry meat products being downgraded due to poor quality and muscle myopathies the use of genetic selection for meat quality traits in turkeys should be considered. Selection for meat quality has been successfully implemented in other livestock species and adaptation of these programs could be applied in turkey breeding. The objective of this study was to estimate the heritabilities and phenotypic correlations of several common meat quality traits to evaluate their selection potential in turkeys.

**Materials and Methods:** Pedigree toms from seven genetic lines were processed at a commercial facility where live weight at slaughter and whole breast muscle weight (weight of both *Pectoralis major* and *minor*) were recorded. *P. major* (fillet) ultimate pH, color (CIELAB values), drip loss, cooking loss, and shear force were also measured. White striping was rated by several recorders using a 1-4 scale. The heritability for each trait was estimated using Gibbs sampling implemented in the BLUPf90 family of programs. Genetic line, hatch week, and slaughter date were included as fixed effects in each univariate model with the addition of recorder as a fixed effect when analyzing white striping score.

**Results:** Moderate positive correlations were observed between breast muscle weight and fillet lightness (L\*), yellowness (b\*), and cooking loss (r = 0.24, 0.20, and 0.18, respectively) showing that larger birds tended to have lighter, more yellow fillets with lower water holding capacity during the cooking process. Moderate negative correlations were observed between ultimate pH and L\*, drip loss, and shear force (r = -0.27, -0.17, and -0.16, respectively). Negative correlations between pH and lightness, drip loss, and shear force are well documented in relation to pale soft exudative (PSE) meat and were expected. The other notable correlation was between breast meat yield (BMY) and white striping score (r = 0.10) showing birds with larger breast muscles relative to body weight tended to have higher white striping scores. High heritability estimates were observed for live weight (h<sup>2</sup> = 0.56; SD = 0.023; N = 19,655), breast muscle weight (h<sup>2</sup> = 0.49; SD = 0.022; N = 19,957), and BMY (h<sup>2</sup> = 0.41; SD = 0.021; N = 19,527) which is typical of growth traits. Ultimate pH (h<sup>2</sup> = 0.30; SD = 0.044; N = 3,181) along with L\* (h<sup>2</sup> = 0.22; SD = 0.019; N = 13,782), a\* (h<sup>2</sup> = 0.27; SD = 0.018; N = 13,782), b\* (h<sup>2</sup> = 0.24; SD = 0.018; N = 13,782), and white striping score (h<sup>2</sup> = 0.19; SD = 0.018; N = 12,826) all showed moderate heritability. The estimates calculated in this study for ultimate pH and fillet colour were similar to previously published estimates in turkeys. Drip loss (h<sup>2</sup> = 0.03; SD = 0.022; N = 3,171), cooking loss (h<sup>2</sup> = 0.09; SD = 0.029; N = 3,221), and shear force (h<sup>2</sup> = 0.07; SD = 0.041; N = 2,620) all had low heritability estimates and were low compared to previous estimates in broilers, pigs, and lambs.

**Conclusion:** The parameters estimated in this study show great potential for the incorporation of meat quality traits in turkey selection programs to improve the overall quality of breast meat and reduce the occurrence of myopathies such as white striping. This study is part of a larger project considering the selection of turkeys for improved meat quality.

Keywords: Gibbs sampling, heritability, meat quality, turkeys, white striping



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### ROLE OF THE CALPAIN-CALPASTATIN SYSTEM IN TENDERNESS OF LONGISSIMUS LUMBORUM IN BRAHMAN AND ANGUS STEERS

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**Objectives:** Proteolysis and tenderness are important focuses for meat quality in Brahman, which tend to be tougher with more variability. Although the calpain-calpastatin system is largely responsible for postmortem tenderization, variability in system's content and activity within the Brahman is not well understood. Therefore, the objectives were 1) determine calpain activity and calpastatin content in the *longissimus lumborum* (LL) of Angus and Brahman, and 2) determine calpastatin content in the LL in tough and tender Brahman.

**Materials and Methods:** For the first study, Brahman and Angus steers (n=14 per breed) were raised together and harvested. Samples of the LL were taken at 1, 3, 6, 24 h and 14 d postmortem, frozen in liquid nitrogen, and stored at - 80°C until further analysis. For calpain activity, samples from all time points were pulverized, diluted with extraction buffer, and homogenized. Then supernatants were plated with a fluorescent substrate. Buffers were added to measure calpain activity with and without calcium added. Calpastatin content of 1 and 24 h samples were evaluated using Western blotting, and the intact band was quantified. Data were analyzed using SAS with fixed effects of breed (b), time (t), and their interaction (b×t). The second study utilized LL from only Brahman steers; samples were sorted into tough (n=11) and tender (n=10) based on trained sensory panel scores of 14d aged steaks. Calpastatin content was evaluated as described for the first study. Data were analyzed with fixed effects of tenderness group (td; tough vs tender), time (t), and their interaction (td×t). Time was used as a repeated measure for both studies.

**Results:** In the first study, Brahman exhibited less calpastatin degradation from 1 to 24h (b×t, P=0.02). Brahman had greater calpastatin content (P<0.05) at 1 and 24h compared with Angus. Calpain activity with added calcium decreased over time (t, P<0.0001), and Angus tended to have higher activity (b, P=0.07) compared with Brahman. Calpain activity without calcium decreased more rapidly in Angus (b×t, P<0.05). In the second study, calpastatin levels at 1h were higher than 24h (P<0.0001). However, neither td group (P=0.37) nor td×t (P=0.97) affected calpastatin content.

**Conclusion:** Increased Brahman calpastatin and less degradation of the protease over time from the first study may suggest more inhibited calpain activity, thus blocking postmortem degradation. This combined with the trend in decreased calpain activity may explain the differences between the tenderness across breeds. However, within the Brahman breed, variable calpastatin levels did not distinguish tough from tender Brahman. Further exploration is needed to fully understand the mechanisms that ultimately decide postmortem tenderness differences in Brahman.

Keywords: calpain-1, calpastatin, tenderness



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#### IMPACT OF MATERNAL NUTRITION ON POSTNATAL GROWTH OF CROSSBRED BEEF STEERS

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**Objectives:** Maternal nutrition of beef cows is critical to programing the fetus for improved performance and meat quality. Cows pastured on range often have reduced forage quality compared to cows on irrigated pasture. Therefore, the objective of this study was to determine the effects of maternal nutrition on the subsequent growth and carcass characteristics of castrated male offspring from multiparous crossbred beef cows that were pastured on irrigated pasture (IRR) vs rangeland (RAN) during early and mid-gestation.

**Materials and Methods:** Twenty-four crossbred steers were weaned from their dams which were pastured on irrigated pasture or rangeland during early and mid-gestation. Twelve steers were from IRR dams and 12 steers were from RAN dams. After weaning steers were placed on a backgrounding diet for four weeks, designed to gain 1.1 kg/d before being transitioned to a finishing ration. Steers remained on the finishing ration until backfat reached 1.02 cm over the 12<sup>th</sup> and 13<sup>th</sup> rib prior to harvest. Weaning weights were adjusted to 205 days of age, post-backgrounding weights/initial feedlot weights, and day 160 feedlot weights were collected. Additionally, average daily gain (ADG) for the backgrounding and feedlot phases were calculated. Data was analyzed using the MIXED procedure is SAS. Significance was determined at *P* < 0.05.

**Results:** Adjusted weaning weights were greater (P = 0.0107) for IRR compared to RAN steers, while initial feedlot weights were not different (P = 0.6943) between the two groups. RAN steers had higher ADG in both the backgrounding (P = 0.0069) and in the feedlot (P = 0.0022) phases. Therefore, on day 60 of the finishing ration RAN steers weighed more than IRR steers (P = 0.0299), 617.8 kg vs 586.0 kg, respectively. The initial data suggests RAN steers are exhibiting compensatory growth post weaning and not only have improved performance during the backgrounding phase but also the finishing phase.

**Conclusion:** Understanding the impact of maternal environment on steer calf performance will provide an opportunity for the industry to produce more acceptable products for consumer consumption.

Keywords: beef, carcass, fetal programming, growth



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#### COLOR QUALITY OF BEEF FROM CATTLE FED HIGH LEVELS OF VITAMIN E FOLLOWING PROLONGED AGING

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**Objectives:** Meat color is the most important factor influencing consumers' initial meat purchasing decisions. In products with prolonged aging times, such as commodity exports, accelerated oxidation can occur, resulting in shortened retail display time and negative flavor attributes. We hypothesized the use of high levels of the antioxidant Vitamin E ( $\alpha$ -tocopherol) may retard oxidation rates in beef aged a prolonged period of time. Our objective was to evaluate the impact of feeding high levels of Vitamin E on meat color quality in beef striploins after prolonged aging compared to beef from the general population.

**Materials and Methods:** Crossbred *Bos taurus taurus* cattle (n=150) across fifteen pens (n=10/pen) were grain-finished on corn-based rations and supplemented with 2,200 International Units (IU) of Vitamin E for the final one hundred days on feed. One low-Choice strip loin was selected from one carcass per pen (n=15). Additionally, low-Choice strip loins (n=15) were selected for controls from the packing plant which harvests locally produced, corn-fed *Bos taurus taurus* cattle. Loins were split into thirds, and each third was randomly assigned to 3, 6, or 9 weeks of postmortem storage in vacuum packages. After aging, loins were cut into one 2.54-cm steak for instrumental and subjective color analysis across 7 days of retail display. Two 1.27-cm steaks were fabricated and halved for lipid oxidation after 0, 4, or 7 days of retail display. Steaks used for retail display were placed on foam trays, overwrapped with an oxygen permeable film, and placed under simulated retail display conditions up to 7 d at 3°C. Thiobarbituric acid reactive substance values (TBARS) were measured on days 0, 4, or 7 of retail display. Instrumental color and Delta E (overall color change over time) values were measured via colorimeter measuring L\* (lightness), a\* (redness), and b\* (yellowness). Subjective discoloration was also evaluated daily during retail display by a panel of five trained panelists using a percentage scale where 0% = no discoloration and 100% = complete surface discoloration.

**Results:** A treatment-by-age-by-retail display interaction (P < .0001) in percent discoloration occurred, as lower discoloration occurred in Vitamin E loins across 3, 6, and 9 weeks of aging. A treatment-by-retail display interaction (P < .0001) occurred for a\* (redness), as Vitamin E loins exhibited greater a\* values across 3, 6, and 9 weeks of aging. This was supported by in delta E values, as control loins had larger delta E values compared to Vitamin E loins (P < 0.05). Vitamin E loins had less (P = 0.03) lipid oxidation compared to control loins at 3, 6, and 9 weeks of aging. Although some differences in the corn-based diets may have occurred, the magnitude of these color differences strongly suggest that feeding high levels of Vitamin E to cattle sustained meat color and oxidative stability during prolonged aging compared to beef obtained from the general population.

**Conclusion:** Results from this study support the feeding of high levels of Vitamin E to ensure color quality of beef undergoing extended aging.

Keywords: discoloration, oxidation, Prolonged aging, Retail display, Vitamin E



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#### THE IMPACT OF FAT CONTENT LABELING ON CONSUMER SENSORY EVALUATION OF 80/20 GROUND BEEF

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**Objectives:** The purpose of this study was to evaluate the effect of providing consumers information related to the fat content of ground beef patties prior to sample evaluation on palatability traits.

**Materials and Methods:** 80% lean/20% fat ground beef chubs (n = 15) from the same production day and lot, were obtained from a commercial source. Individual chubs were randomly assigned to one consumer panel session and patties were randomly assigned to 1 of 6 different labeled fat contents: 90% lean/10% fat (**90/10**), 80% lean/20% fat (**80/20**), 73% lean/27% fat (**73/27**), plus 2 treatments labeled as "lean" or "extra lean", as well as an unlabeled treatment (**NONE**). Ground beef was formed into 151.2 g (approximately 13 cm diameter; 1 cm thick) patties using a commercial patty press 11 days after manufacture. All patties were cooked to a medium (71°C) degree of doneness on a clamshell grill. Before sampling, consumers were provided information about the informed fat content, but information was withheld for the NONE sample. Samples were evaluated by consumers (n = 105) for juiciness, tenderness, texture, flavor liking overall liking and purchasing intent. Consumers also rated each sample as acceptable or unacceptable within each trait. A 0-to-100-point continuous line scale was used for each trait evaluation and rating. Percent change of consumer ratings from the NONE sample were found utilizing the equation:(consumer trait scores – consumer NONE scores)/consumer NONE scores. A completely randomized design was used for data analysis.

**Results:** There were no differences (P > 0.05) for tenderness, flavor liking, texture liking, overall liking, or purchasing intent among treatments. For juiciness acceptability, 90/20 was rated lower (P < 0.05) than 80/20, 73/27, and NONE, but was similar (P > 0.05) to the lean and extra lean samples. There was no difference (P > 0.05) in the percent change in ratings among the fat treatments across all palatability traits. When evaluating tenderness acceptability, 80/20, 73/27, and NONE samples had a greater (P < 0.05) percentage of samples rated acceptable for juiciness compared to 90/10. Lean and extra lean samples were similar (P > 0.05) to 90/10 for the percentage of samples rated acceptable for juiciness. There were no differences (P > 0.05) among treatments for the percentage of samples rated acceptable for flavor, texture, or overall acceptability. When evaluating the change in acceptability ratings due to labeling, there were no differences (P > 0.05) among treatments for flavor, texture, and overall acceptability. However, labeling with fat percentage had less (P < 0.05) of an impact on tenderness acceptability for extra lean as compared to 90/10, 80/20 and 73/27 samples. Fat labeling resulted in less (P < 0.05) change in the percentage of samples rated acceptable for juiciness for extra lean and 90/10 samples than 80/20 and 73/27 samples.

**Conclusion:** Ground beef samples with high fat labels resulted in increased juiciness ratings as well as a higher percentage of patties rated acceptable for juiciness. However, the reverse was not observed for lower fat percentage samples nor samples labeled as lean or extra lean. This provides evidence that consumer eating perceptions are influenced by fat percentage labeling, but the impact is greater with higher fat products.

Keywords: consumer, fat content, ground beef, lean, palatability



### Meat and Poultry Quality

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#### CHARACTERIZING GROUND BEEF THROUGHOUT VARIOUS STORAGE LENGTH INTERVALS FOLLOWED BY SIMULATED RETAIL DISPLAY

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**Objectives:** The objective of this study was to conduct two separate experiments to characterize shelf-life characteristics of ground beef formulations throughout various intervals of dark storage followed by retail display under different lighting conditions.

Materials and Methods: In experiment 1, ground beef in clear chub packaging in two lean: fat formulations (81:19 and 73:27) were procured from a federally inspected commercial processor. Ground beef packages were stored in darkness for intervals of 7, 11, 15, 19, 23 and 27 days post-grinding. At each storage interval, packages of the same lean formulation were blended together. Approximately 454 g of each ground beef blend were portioned onto polystyrene trays and overwrapped with PVC film. Trays were randomly allocated to initial analysis or assigned to continuous Fluorescent or LED lighting for 36 or 72 hours of simulated retail display in a refrigerated room at -1.0 to 1.0 °C with no additional ambient light. In experiment 2, beef trimmings were course and fine ground to produce 454±20 g ground beef (approximately 85:15 lean:fat) rollstock packages. Package seals were evaluated prior to randomly subjecting packages to dark storage, full light (continuous fluorescent or LED) or intermittent light (12 hours of fluorescent or LED light exposure followed by 12 hours of dark storage) for 6, 12 or 18 days post-grinding. Objective color (L\*, a\*, b\*) scores, aerobic bacterial counts and lipid oxidation (TBARS) were evaluated at each storage or display interval in both experiments. In experiment 2, an additional set of objective color scores were recorded after packages of ground beef were exposed to oxygen and allowed to bloom for at least 20 minutes on each microbiological sampling day. Results: The interaction between storage time (day 7, 11, 15, 19, 23, or 27 days of dark storage), display time (initial analysis or 72 hours) and lean formulation (81:19 or 73:27) was significant (P < 0.05) for a\* values, indicating that a\* values decreased with increased dark storage and display time, and lower a\* values were detected from the 73:27 lean formulation throughout all sampling times. However, source of light (LED or Fluorescent) did not impact L\* or a\* values among ground beef in experiment 1. Aerobic bacterial counts increased (P < 0.05) between early and late storage intervals and due to display time. In experiment 2, packages of ground beef stored for 12 days post-grinding that were allowed to bloom had higher (P < 0.05) L\* values and lower (P < 0.05) a\* and b\* values than packages sampled at 6 or 18 days post-grinding and allowed to bloom at final sampling. Lower (P < 0.05) a\* values resulted among packages stored in intermittent light after exposure to oxygen at final sampling.

**Conclusion:** These results suggest that storage interval, display time and lean formulation influence ground beef characteristics in continuous intervals of storage. This information will be beneficial for future replications and additional work evaluating changes in ground beef throughout storage.

Keywords: ground beef, objective color, shelf-life characteristics



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#### EVALUATION OF CARBOXYMYOGLOBIN FORMATION IN GROUND BEEF WITH DIFFERING FAT PERCENTAGES

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**Objectives:** Meat color is one of the most important visual attributes consumers use when purchasing fresh beef. Myoglobin in the form of oxymyoglobin (OxyMb) and carboxymyoglobin (COMb) results in a bright cherry-red color, desired by consumers. COMb denatures slower during the cooking process than OxyMB; thus, persistent pinking of cooked meat can result. The objective of this study was to evaluate COMb formation and persistent pinking differences in ground chuck and ground round patties.

**Materials and Methods:** Two USDA Choice (IMPS 116) chuck rolls and two USDA Choice inside rounds (IMPS 184) were collected from a commercial packing facility. The sub-primals were wet aged for 12 d. Cuts were cubed and ground separately to produce ground round and ground chuck. Proximate analysis was conducted to ensure target lean to fat ratios of ground round (90:10) and ground chuck (80:20). Ground beef from each anatomical location was separately formed into 225 g patties. Patties were placed onto foam trays (n = 48 patties) with an absorbent pad and over-wrapped with polyvinylchloride film (PVC). Trays were randomly assigned to 16 master bags (MB) with 3 trays each. Twelve MB were flushed with a 20% CO<sub>2</sub>, 0.4% CO, 79.6% N (Trigas) blend with the remaining 4 bags sealed with ambient air as controls. Bags were placed in dark storage until opening at 24 h and 8 d. Patties stored for 8 d in dark storage were then placed in simulated retail display for 2 d (10 d total before analysis). Before opening each MB, headspace analysis was conducted to determine percentage CO<sub>2</sub>, CO, and O<sub>2</sub> remaining. Upon opening, HunterLab measurements were taken on all patties; 2 patties were cooked to evaluate persistent pinking and 1 patty was used for thiobarbituric acid reactive substances (TBARS) analysis. The formation of COMb was confirmed by measuring ratio of absorbance at 543 nm/581 nm. Patties were cooked to an internal temperature of 71°C and placed on ice for 5 min to reduce post-cooking temperature rise. Patties were then removed and cut parallel to surface to measure color with HunterLab (3 readings per patty).

**Results:** Raw patties from control MB regardless of anatomical location were less red (P < 0.05) than patties flushed with the Trigas blend. Patties in dark storage for 8 d and retail for 2 d had greater COMb formation than patties stored for 24 h. Also, on d 10 (2 d retail display) uncooked ground round patties were redder (higher a\*; P < 0.05) than the ground chuck patties. At 24 h, all raw patties were lighter (higher L\*; P < 0.05) than patties stored d 10 (8 d dark storage and 2 d retail display). On d 10 raw patties flushed with the Trigas blend were significantly (P < 0.05) lighter than patties packaged with ambient air. Cooked patties stored 10 d were redder (P < 0.05) than patties stored 24 h. Cooked ground chuck patties on d 10 were darker (lower L\*; P < 0.05) than ground round patties and ground chuck patties cooked after 24 h storage. Lipid oxidation of patties was higher (P < 0.05) in patties stored 10 d compared to 24 h.

**Conclusion:** Patties flushed with Trigas blends forming stable COMb cause increased persistent pinking and can lead to consumer confusion when cooking these patties. Thus, additional research should be conducted to better understand the relationship of lean:fat ratios and dark storage times resulting in increased persistent pinking.

Keywords: Carboxymyoglobin, Meat color, Persistent pinking



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#### COLOR AND LIPID STABILITY, FREE AMINO ACID PROFILE, AND FLAVOR OF WET AND DRY-AGED BEEF LOINS FROM CATTLE FED ULTRA-HIGH DOSES OF VITAMIN E

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**Objectives:** Dry-aged beef has unique flavor, partly because of prolonged exposure to oxygen and lipid oxidation. The experiment tested the hypothesis that feeding ultra-high doses of vitamin E would reduce lipid oxidation and stabilize color during dry aging and retail display and support the development of desirable flavors compared to the general population of fed beef.

**Materials and Methods:** Cattle (n = 150; 10/pen) were grain-finished with the dietary addition of 2,200 IU of vitamin E ( $\alpha$ -tocopherol) per head per day. One low-Choice carcass was selected per pen (n = 12). Low Choice control carcasses (n = 12) were randomly selected from commercial, fed-cattle production (which are typically fed ca. 50 IU/day). Strip loins within vitamin E and control treatments were randomly assigned to wet or dry aging for 42 days. After aging, the longissimus lumborum muscle of a 1.27 cm steak from each loin was isolated and cut into equal sized rectangles. One half was immediately frozen at -80 C while the other was subjected to retail display conditions for 8 days while covered with an oxygen-permeable, polystyrene film. Lipid oxidation was measured using thiobarbituric acid reactive substances (TBARS) on day 0 and 42 of aging and after 8 d of retail display. Free amino acids were measured on day 0 and day 42 of aging. Percentage discoloration during retail display was rated daily. A colorimeter measured L\*, a\*, and b\* values using D65 illumination, 2-degree standard observer, and an 8 mm aperture. Data were analyzed as a 2 x 2 factorial with repeated measures over time. Least square means were determined by day, with significance noted at a *P* < .05 and a Tukey-Kramer adjustment for multiple comparisons.

**Results:** On day 0, control and treatment loins did not differ in lipid oxidation (P = 0.936). On day 42, control dry aged and control wet aged loins had the highest TBARS values and vitamin E wet and dry aged loins had lower TBARS values (P = 0.043). After 8 days of retail display post-aging, control dry aged and control wet aged steaks had the highest TBARS values and vitamin E wet and dry aged steaks tended to have lower TBARS values (P = 0.085). Free amino acids related to positive beef flavor attributes (such as leucine, isoleucine, valine, glycine, and glutamic acid) were higher (P < .05) for samples dry aged for 42 days than wet-aged samples. Trained sensory panelists generally noted more positive flavor notes and fewer negative flavor notes in dry-aged beef from vitamin E steaks compared to dry-aged control steaks. There was a three-way interaction for discoloration between treatment (control vs vitamin E), aging type (dry vs wet aging) and retail display day (P < 0.0001). Wet-aged controls discolored fastest, followed by dry-aged controls and wetaged vitamin E samples. Dry-aged vitamin E samples had the lowest discoloration. There were aging by–day-and agingby-treatment interactions for a\* values (P > .0001 and P = 0.0104, respectively). Generally, vitamin E-treated samples sustained higher redness values for longer times.

**Conclusion:** Dietary supplementation of ultra-high levels of vitamin E can decrease the negative flavors produced by oxidation while maintaining the unique flavor characteristics of dry-aged beef, minimizing oxidation, and stabilizing color.

Keywords: Color, Dry Aging, Free amino Acids, oxidation, Vitamin E



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#### THE EFFECT OF GRAZING BOTANICALLY DIVERSE PASTURE ON THE FATTY ACID PROFILE AND VITAMIN E CONTENT OF BEEF.

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**Objectives:** A desire for more biodiverse and sustainable pasture-based feeding systems for ruminants has led to growing interest in the use of botanically diverse pasture to improve the nutritional profile of beef. The aims of the study were to determine the impact of grazing three different pasture types on the fatty acid profile and vitamin E ( $\alpha$ -tocopherol and  $\gamma$ -tocopherol) content of beef.

**Materials and Methods:** Hereford x Holstein Friesian male calves (n = 60) were assigned to one of three grazing treatments (n = 20 per treatment); a monoculture (PRG) (perennial ryegrass (*Lolium perenne*)), a two-species pasture (perennial ryegrass and white clover (*Trifolium pratense*)) (PRG+WC) and a mixed species (MS) pasture (perennial ryegrass, timothy (*Phleum pratense*), white clover, red clover (*Trifolium repens*), chicory (*Cichorium intybus*) and plantain (*Plantago lanceolata*)). Following slaughter and postmortem ageing,  $\alpha$ -tocopherol and  $\gamma$ -tocopherol concentrations were determined by high performance liquid chromatography and fatty acid methyl esters by gas chromatography. Statistical analysis was performed in R. Statistical analysis involved one-way ANOVA followed by Tukey's *post-hoc* test. **Results:** Total polyunsaturated fatty acid (PUFA) proportions were higher (P < 0.01) in the MS beef compared to the PRG and PRG+WC beef. The ratio of PUFAs to saturated fatty acids (SFA) and *n*-6 to *n*-3 PUFAs were higher (P < 0.01) for the MS beef compared to PRG and PRG+WC beef.  $\alpha$ -Tocopherol concentration was higher (P < 0.05) in muscle from PRG beef compared to beef from MS animals, but PRG+WC beef was not significantly different from either.

**Conclusion:** Grazing animals on botanically diverse pasture affected the fatty acid profile and vitamin E content of the meat. MS beef has a higher proportion of PUFAs and a lower concentration of  $\alpha$ -tocopherol, which may result in meat that is more prone to oxidation and quality deterioration.

Keywords: Beef, Fatty acids, Nutritional composition, Pasture-fed, Vitamin E



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**RELATIONSHIP OF BEEF CARCASS YIELD ATTRIBUTES TO QUALITY GRADE OUTCOMES** 

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**Objectives:** Carcass yield attributes are assumed to be strongly associated with quality grade. Carcass data from individual animals (n = 644,745) collected by the Beef Carcass Research Center from 1992 to 2021 was utilized to quantify the relationships between carcass yield attributes and the probability of a carcass grading USDA Choice or better.

Materials and Methods: Yield attributes included hot carcass weight (HCW), ribeye area (REA), 12<sup>th</sup> rib subcutaneous fat thickness (FAT), calculated yield grade (YG), and hot carcass weight to ribeye area ratio (RATIO). Logistic regression analyses were used to calculate the probability of the binary response variable of carcasses grading Choice or better (yes or no). Linear regression was used to determine specific points at which a carcass reaches USDA Choice. **Results:** Hot carcass weight was linearly (P < 0.01; c = 0.57) associated to the probability of carcasses grading Choice or better. The average carcass reached USDA Choice at 283.2 kg; a 1% change in probability of grading Choice occurred concomitant with each 7.7 kg change in HCW. Backfat was also strongly associated (P < 0.01; c = 0.67) in a curvilinear manner to the probability of a carcass grading Choice or better. On average, a carcass achieved USDA Choice at 0.91 cm of 12<sup>th</sup> rib subcutaneous fat depth. Between 0 and 2 cm of 12<sup>th</sup> rib subcutaneous fat, a 1% increase in the probability of grading Choice occurred with the addition of 0.0357 cm of backfat. However, the increase in grading Choice or better slowed in relation to subcutaneous fat accrual between 2 and 3 cm of 12<sup>th</sup> rib fat depth; a 1% increase in Choice or better grading required the addition of 0.0667 cm of backfat. The relationship of quality grade and the primary carcass metric of muscling, REA, is known to be antagonistic in nature. As REA increased, the probability of grading USDA Choice or better decreased; a 1% change in probability of grading Choice occurred with a 0.39 cm<sup>2</sup> change in REA. The probability of achieving Choice or better by YG was represented by a positive quadratic relationship (P < 0.01; c statistic = 0.68); the average carcass achieved USDA Choice at a yield grade of 2.29 and accrued 1% increase in Choice carcasses with each additional 0.0723 units of yield grade. The probability of a carcass grading Choice or better decreased as the RATIO increased. According to the expected RATIO reported on a USDA dot grid, a carcass is required to have 11.81 cm<sup>2</sup> of REA for each 45.35 kg of HCW. A carcass with a RATIO of a 1.83, the standard expected from the USDA REA: HCW relationship used in yield grading, had a 52% probability of grading Choice or better, whereas a 1% change in Choice or better was caused by a 0.0274 unit change in RATIO.

**Conclusion:** These data support the knowledge that carcass fat attributes are strongly associated with quality grade outcomes and further supports the antagonistic relationship between carcass muscling and fat deposition.

Keywords: carcass, fat, quality, ribeye area, yield



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PREDICTION OF COLOR STABILITY OF BONELESS PORK CHOPS USING EARLY AND AGED QUALITY TRAITS

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**Objectives:** Meat discoloration is known to be related to some quality traits, such as ultimate pH, water holding capacity, and color. Because of this relationship, quality traits may be predictive of the amount of discoloration that develops during retail display. Such relationships might allow for selection to improve color stability of pork. Therefore, the objectives were to use early and aged pork quality traits to develop regression models for the prediction of instrumental and subjective pork discoloration over time.

**Materials and Methods:** Boneless loins were collected from 468 pigs in multiple groups. Early quality measurements were collected on the ventral face of 202 loins at the approximate location of the  $10^{th}$  rib at 1 d postmortem. Ultimate pH, CIE L\*, a\*, and b\*, and visual NPPC color, marbling, and subjective firmness were evaluated. These same traits were collected on all loins after 11 or 14 d of postmortem aging with the addition of purge loss. Then, a 2.54 cm chop was cut from each loin at the approximate location of the  $10^{th}$  rib for retail display. Chops were individually placed on  $13 \times 13$  cm Styrofoam trays, overwrapped using oxygen-permeable polyvinylchloride film, and displayed under constant lighting for 7 d. On each day of display, visual discoloration and instrumental color measurements were recorded. Visual discoloration was evaluated using a 10-cm line scale anchored at 0, 50, and 100% discoloration. A Hunter spectrophotometer was used to collect reflectance data, which was used to calculate 630/580 nm ratio and Metmyoglobin (MMb) content. The REG procedure of SAS was used to develop prediction models for changes (d7 – d1) in 630/580 nm ratio, MMb content, and visual discoloration percentage using a stepwise selection method and early or aged traits as independent variables.

**Results:** From d1 - d7 of display, changes in 630/580 nm ratio values ranged from |0.35| to |1.66| units, changes in MMb content ranged from 2.28 – 40.56%, and changes in visual discoloration scores ranged from 0 – 54%. Early quality traits were able to predict 33% (R<sup>2</sup> = 0.33) of variation in 630/580 nm ratio, 36% (R<sup>2</sup> = 0.36) of variation in MMb content, and 44% (R<sup>2</sup> = 0.44) of variation in subjective discoloration score over time. Aged quality traits were able to predict 63% (R<sup>2</sup> = 0.63) of variation in 630/580 nm ratio, 61% (R<sup>2</sup> = 0.61) of variation in MMb content, and 37% (R<sup>2</sup> = 0.37) of variation in subjective discoloration (R<sup>2</sup> = 0.30), while are explained the greatest proportion of variation in MMb content (partial R<sup>2</sup> = 0.32) and subjective discoloration (partial R<sup>2</sup> = 0.30), while are explained the most variation in 630/580 nm ratio (partial R<sup>2</sup> = 0.37) and MMb content (partial R<sup>2</sup> = 0.41) while L\* explained the majority of variation in subjective discoloration (partial R<sup>2</sup> = 0.37) and MMb content (partial R<sup>2</sup> = 0.41) while L\* explained the majority of variation in subjective discoloration (partial R<sup>2</sup> = 0.37) and MMb content (partial R<sup>2</sup> = 0.41) while L\* explained the majority of variation in subjective discoloration (partial R<sup>2</sup> = 0.37) and MMb content (partial R<sup>2</sup> = 0.41) while L\* explained the majority of variation in subjective discoloration (partial R<sup>2</sup> = 0.37) and MMb content (partial R<sup>2</sup> = 0.41) while L\* explained the majority of variation in subjective discoloration (partial R<sup>2</sup> = 0.37) and MMb content (partial R<sup>2</sup> = 0.41) while L\* explained the majority of variation in subjective discoloration (partial R<sup>2</sup> = 0.25). Increases in redness and pH resulted in decreased discoloration over time, while increases in L\* and purge loss resulted in increased discoloration.

**Conclusion:** Both early and aged traits were able to predict the extent of discoloration in pork chops. Aged traits were more predictive of instrumental measures (MMb content, 630/580 nm ratio), while early traits were more predictive of subjective discoloration. Overall, loins that were darker, redder, and had greater pH values had better color stability over time.

Keywords: color, color stability, discoloration, Pork, prediction



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**EVALUATING THE EFFECTS OF STANDARDIZATION METHODS ON NIX PRO AND HUNTERLAB READINGS** A. Carlock <sup>1,\*</sup>, M. Denzer <sup>1</sup>, M. Pfeiffer <sup>1</sup>, G. G. Mafi <sup>1</sup>, R. Ramanathan <sup>1</sup> <sup>1</sup>Animal and Food Sciences, Oklahoma State University, Stillwater, United States ranjith.ramanathan@okstate.edu

**Objectives:** Meat color is considered the most important quality attribute that influences consumer purchasing decisions. Handheld spectrophotometers such as HunterLab (HL) or Minolta are used to objectively measure meat color. Recently the Nix Color Sensor Pro has been tested in meat color research because of its inexpensiveness and user-friendly interface. Prior to measurement, the HL is standardized with manufacture recommendations. However, no standardization step is involved in Nix color measurements. Previous research focused on bloomed steaks to determine the correlation between Nix and HL. However, limited knowledge is available on how Nix captures color changes from red to brown. Therefore, the first objective of this study was to investigate the correlation between the HL and Nix for  $a^*$  color measurements with different types of standardization. The second objective was to determine the ability to quantify discoloration over time using Nix and HL.

**Materials and Methods:** Eight USDA Low Choice short loins were collected from a beef processor five days postmortem. The *longissimus lumborum* (LL) and *psoas major* (PM) muscles were separated from short loins and were sliced into 1.9 cm thick steaks (n = 8). For objective one, four standardization approaches with the HL were assessed: no standardization through polyvinyl chloride overwrap (PVC), standardization through PVC, no standardization through vacuum packaging (VP), and standardization through VP. For retail comparisons, standardization through PVC was evaluated and for research comparisons standardization was through VP. Steaks were allowed to bloom for 1 hour, then packaged in PVC and held in coffin-style retail cases for display period. For objective 2, LL color was measured on display days 0, 3, 6, and 8. For the PM, color was measured on display days 0, 2, and 4. CIE *a*\* was measured on each instrument and standardization combination for each muscle and display day to determine correlation and quantify discoloration. Proc Univariate, Proc Corr (r), and linear best fit (R<sup>2</sup>) were determined using SAS separately for LL and PM. The standardization method with the best fit was utilized for further analysis.

**Results:** There were effects of standardization approach on color readings. LL steak color readings were taken after standardizing and through VP film resulted in the best fit ( $R^2 = 0.86$ ) and correlation of r = 0.93 between the two instruments on d 0. However, the best fit and correlation decreased for LL color readings taken through VP with the transition from bright red to brown with storage time. On d 8, color readings taken on LL steaks with no standardization of the HL but read through PVC increased best fit and correlation. The PM for both instruments reading through VP film without standardizing the HL resulted in the best fit and correlation on d 0 and 4 than d 2 (d 4 > d 0). However, during the transition from red to brown the best fit and correlation were lower for color readings taken through VP film. These results indicate the Nix is less sensitive in quantifying color changes during retail display.

**Conclusion:** The user-friendly nature of Nix will allow for its potential use to determine color changes under retail or industry conditions. The current research indicates that if both Nix and HL are used, researchers may need to take precautions in the standardization step to get comparable data.

Keywords: meat color, HunterLab, NIX, beef color



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OHIO BEEF QUALITY AUDIT – 2021: SURVEY OF CARCASS CHARACTERISTICS RELATED TO QUALITY ATTRIBUTES B. M. C. Garcia<sup>1,\*</sup>, S. Boyles<sup>2</sup>, J. Rumble<sup>3</sup>, B. Bohrer<sup>1</sup>, A. Relling<sup>2</sup>, L. G. Garcia<sup>1</sup> <sup>1</sup>Animal Science / Meat Science, <sup>2</sup>Animal Science, <sup>3</sup>Agriculture Communication Education Leadership, The Ohio State University, Columbus, United States garcia.951@osu.edu

**Objectives:** The National Beef Quality Audit (NBQA) was developed to "benchmark" the status of the US beef industry. Since the first NBQA in 1991, much progress has been made in beef production systems fulfilling beef packing plants' needs demanded by consumers. To date, a beef quality audit has yet to be conducted in individual states, such as Ohio. With an estimated number of 300 federally and state inspected meat plants, the collection of beef carcass data could serve to assist beef producers in modifying their livestock practices to reach their end goal. Therefore, the objective is to audit various small-scale meat processing plants to provide a benchmark of the beef industry in Ohio.

**Materials and Methods:** Ohio meat processing plants (n=16) slaughtering beef carcasses were selected to contribute data of beef carcass characteristics. Beef carcasses (n=371) included in the audit were ribbed between the 12<sup>th</sup> and 13<sup>th</sup> ribs and allowed to bloom for 20-minutes. Carcass characteristics were evaluated for yield grade (YG) (back fat thickness (cm.), loin muscle area (cm<sup>2</sup>), kidney, pelvic and heart fat (%), and hot carcass weight (kg.)), quality grade (QG) factors (skeletal maturity, lean maturity, and marbling score) and external fat color. Tools used for evaluation include a USDA PYG Ruler (back fat thickness), ribeye dot grid (loin muscle area), USDA Marbling Cards (marbling scores) and a color reference guide. Statistical analysis included arithmetic means by MEANS SAS procedure, least squares means and standard error of the mean by PROC GLIMMIX SAS procedure, and frequency distributions by Excel.

**Results:** Mean yield grade factors were backfat thickness: 1.1 cm, loin muscle area: 82.0 cm<sup>2</sup>, kidney, pelvic, and heart fat: 1.6%, and hot carcass weight: 343.1 kg. The mean yield grade was 2.6 with frequency distributions of 21.3% YG 1, 48.5% YG 2, 23.4% YG 3, 5.6% YG 4, and 1.4% YG 5. Mean quality grade factors were: skeletal maturity: A<sup>89</sup>, lean maturity: A<sup>83</sup>, and marbling score: Small<sup>55</sup>. The mean QG was Select<sup>43</sup> with frequency distributions of 5.4% Prime, 62.6% Choice, 17.5% Select, 6.7% Standard, and 8% other (USDA Commercial, Utility, Cutter, or Canner). External fat color resulted in the mean 2.3 (Creamy White).

**Conclusion:** The in-progress Ohio Beef Quality Audit – 2021 currently demonstrates similarities to beef characteristics at the national level. As the first of its kind at a state level, more is to consider for future audits. The current audit primarily serves as a starting point in collecting beef carcass data to assist beef producers better understand what is produced. This understanding could lead to modifying livestock practices to better fit the needs of the Ohio beef industry. However, more research and data collection are warranted to accurately compare the Ohio beef industry to that of the United States (OBQA vs. NBQA 1991-2016).

Keywords: beef, beef grading, NBQA, Ohio



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**VOLATILE AROMA COMPOUNDS OF GRILLED CHOPS FROM LAMBS FED NOVEL HIGH-ANTHOCYANIN CORN COBS** M. E. Hawkins <sup>1,\*</sup>, C. R. Kerth <sup>1</sup>, D. G. Quadros <sup>2</sup>, T. R. Whitney <sup>2</sup>, W. Xu <sup>3</sup>

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**Objectives:** Oxidative stability is important to improve meat flavor. It was hypothesized that feeding high-anthocyanin (Hi-A<sup>™</sup>; anthocyanin is an antioxidant) corn cobs as roughage to thirty Rambouillet ewe lambs would improve volatile aroma compounds.

**Materials and Methods:** The experiment consisted of three treatments, Hi-A (an experimental variety) corn cob, Low-A (commodity grade) corn cob, or Hay (Bermudagrass) diets, with ten animal replications each. Each experimental diet contained 20% roughage (High-A, Low-A, or Hay) and 80% concentrate which included cottonseed meal, corn, molasses, and minerals. After a 63-d finishing period, the lambs were harvested, and the carcasses were chilled for 14 d (4°C). Boneless loin chops, 2.5 cm thick, were fabricated, vacuum packaged, and frozen (-10°C) until analyzed. Chops were thawed (4°C) for 24 h then placed on a 204°C flat top grill. When the chops reached an internal temperature of 35°C they were flipped and removed when they reached 70°C internally. The chops were immediately cut into 11 cm cubes and 5g were placed in a 20 mL glass vial, while the chop was still hot, where the headspace was collected by SPME for 30 min. Each SPME was desorbed then analyzed using GC/MS. Volatile aroma compounds were expressed as log<sub>n(x+1)</sub> total ion counts area under the curve. Data were analyzed by ANOVA for a completely randomized design with diet as the fixed effect and animal as a random effect with an alpha of 5%.

**Results:** Five volatile compounds were affected by the diets. Both 2-butanone (fruity-green aroma) and 2,3-butanedione (buttery aroma) tended (P = 0.07 and 0.05, respectively) to be greater in chops from lambs fed the Hay diet than those fed the Hi-A corn cob diet; neither of which differed (P > 0.05) from lambs fed the Low-A diet. The compound 2-propanone (pungent aroma) was greater (P = 0.01) in chops from lambs fed the Hay diet than those fed either the Low-A or Hi-A diet, which were similar (P > 0.05). Both 3-methyl-butanal (malty aroma) and methyl benzene (sweet aroma) were lower (P = 0.01 and 0.02, respectively) in chops from lambs fed the Hi-A diet than those fed either the Hay or Low-A diets, which were similar (P > 0.05).

Image:



Diet	2-butanone <sup>1</sup>	2-propanone <sup>1</sup>	2,3- butanedione <sup>2</sup>	3-methyl- butanal <sup>3</sup>	Methyl benzene <sup>2</sup>
Diet	2-outanone	2-propatione	outaneutone	Uutanai	benzene
Hay	13.2ª	14.2ª	11.1ª	14.0 <sup>a</sup>	7.9ª
Low-A	8.6 <sup>ab</sup>	13.5 <sup>b</sup>	6.7 <sup>ab</sup>	13.7 <sup>a</sup>	7.8ª
Hi-A	5.9 <sup>b</sup>	13.3 <sup>b</sup>	5.5 <sup>b</sup>	12.6 <sup>b</sup>	1.0 <sup>b</sup>
SEM	2.16	0.21	1.62	0.32	1.93
P-value	0.07	0.01	0.05	0.01	0.02

**Table 1.** Least squares means of volatile aroma compounds of grilled lamb chops fed Hi-A corn cobs, Low-A corn cobs or Hay diets

<sup>1</sup>Lipid degradation product.

<sup>2</sup>Maillard reaction product.

3Strecker aldehyde.

<sup>ab</sup>Least squares means within a column with different superscripts are statistically different (P < 0.05).

**Conclusion:** The results suggested that feeding lambs Hi-A corn cobs altered the composition of volatile compounds in the boneless lamb loin chops. Volatile aroma compounds perceived as fruity-green, buttery, pungent, malty, and sweet were more apparent in chops from lambs fed either the positive or negative control diets suggesting that feeding lambs a Hi-A corn cobs diet high could improve the cooked aroma in boneless loin chops.

Keywords: Anthocyanin, antioxidants, Lamb Flavor, Volatile flavor compounds



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EARLY POST-MORTEM PH DECLINE RATE IN THE PORK LOIN SIGNIFICANTLY CORRELATES WITH ULTIMATE PH N. Matthews <sup>1,\*</sup>, B. Fields <sup>1</sup>, A. Sosnicki <sup>1</sup> <sup>1</sup>Applied Meat Science, PIC North America, Hendersonville, United States neal.matthews@genusplc.com

**Objectives:** Most pH decline curve (PHDC) work has focused on intervals of 1 hour or more post-mortem and much of this research has been conducted with conventional carcass chilling (minimum temperature > -5°C) combined with electrical stunning. We believe the PHDC in commercial production systems with group pig movement  $CO_2$  stunning and blast chilling (minimum temperature < -10°C) may be very different than that presented in the literature. The focus of this research was to measure PHDC in shorter time intervals in commercial slaughter plants to determine the shape of the PHDC and determine the intervals where pH decline rate ( $\Delta$ pH; rate of pH decline per hour) is crucial for development of ultimate pH (pHu; 20-h).

**Materials and Methods:** Loin ( $\approx$ 10th rib) pH and temperature were collected in 32 carcasses from 4 different plants (n = 6-12/plant). Reed Instruments SD-230 Dataloggers were fitted with Reed TP-07 temperature probes and Hanna FC200B pH electrodes allowing for data collection in 1-min intervals starting at 40-min post-mortem (PM). Data points from every 5-min (40 to 120 min PM), 15-min (45 to 300 min PM and 40 to 55 min PM), 30-min (1 to 10 h PM) and 60-min (1 to 20h PM) were used to determine  $\Delta$ pH for each 5, 15, 30, and 60-minute intervals. Data were analyzed using the PROC CORR procedure of SAS to determine correlations. Regression models for determining pHu were generated using the PROC STEPWISE procedure of SAS and MAXR selection with independent variables including all  $\Delta$ pH variables as well as 40 min pH (pHi). Only models with all variables significant at P < 0.10 were deemed valid.

**Results:** The pHi averaged 6.37 (6.03 to 6.85) and 20-h pH averaged 5.79 (5.43 to 6.02). All times of pH measurement were correlated with 20-h pH (P < 0.001) with r = 0.70 at 40 min PM, linearly decreasing until 75-min PM (r = 0.55), then began to increase until reaching r = 0.99 from 15 to 19h PM. The average pH decline curve generated from these data is different from pH curves using 1 or 2-h intervals. The main differences occur during the first 4-h PM with many of the  $\Delta$ pH at 15-min and 30-min time intervals being very slow to no decline or even slightly increasing in pH. Regression models for 1 (R<sup>2</sup> = 0.49), 2 (R<sup>2</sup> = 0.73), 3 (R<sup>2</sup> = 0.80), 4 (R<sup>2</sup> = 0.86), 5 (R<sup>2</sup> = 0.90), 6 (R<sup>2</sup> = 0.95), 7 (R<sup>2</sup> = 0.97), 8 (R<sup>2</sup> = 0.98), 9 (R<sup>2</sup> = 0.99), and 10 (R<sup>2</sup> = 0.99) variable models (VARM) were generated for determining pHu (all independent variables were significant in the models (P < 0.05). All models included pHi and the only 5-min interval variable used was  $\Delta$ pH45/50 min in the 3, 4, 5, and 6 VARM. After the 5 VARM, the models used more 15-min  $\Delta$ pH than any other time interval. No model contained more than one 30-min  $\Delta$ pH variable. The 60-min  $\Delta$ pH variables were not included in any model before the 6 VARM and no more than 3 times in a single model. The majority of  $\Delta$ pH variables occurred before 5-h PM. The exceptions included  $\Delta$ pH 330 to 360 (3, 4, and 5 VARM),  $\Delta$ pH 480 to 540 (6, 7, 8, 9, and 10 VARM), and  $\Delta$ pH 900 to 960 (10 VARM).

**Conclusion:** These data indicate that determining  $\Delta pH$  in 15-minute intervals is important in explaining variation in ultimate pH and better quantifying the shape of the pH curve in modern commercial facilities using CO<sub>2</sub> stunning with blast chilling systems. Future work will include evaluation of the  $\Delta pH$  time intervals that contribute to the development of pork color, firmness, and water-holding capacity.

Keywords: decline rate, pH, pork, quality



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PREDICTABILITY OF ULTIMATE PH IN PORK LOIN BASED ON POST-MORTEM TEMPERATURE DECLINE

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**Objectives:** It is accepted that post-mortem (PM) temperature decline rate in pig longissimus muscle influences ultimate pH (pHu; 20-h). We are unaware of any work to use individual temperature (TMP) along the TMP decline curve to predict pHu, and thus precisely modify carcass chilling to attain a desired pHu. This research was conducted to determine if loin TMP PM can predict pHu.

**Materials and Methods:** Loin ( $\approx$ 10th rib) pH and TMP were collected in 32 carcasses from 4 different harvest plants (n = 6-12/plant). Reed Instruments SD-230 Dataloggers were fitted with Reed TP-07 TMP probes and Hanna FC200B pH electrodes allowing for data collection in 1-min intervals starting at 40-min post-mortem (PM). Data points from every 5-min (40 to 120 min PM), 15-min (120 to 300 min PM), 30-min (5 to 10 h PM) and 60-min (10 to 20h PM) were used for correlation and prediction equation analysis. The 40-min pH (pHi) was included in prediction equations as it explains the variability in pHu due to peri-mortem stress before chilling. Correlations were determined using the PROC CORR procedure of SAS. Regression models for determining pHu were generated using the PROC STEPWISE procedure of SAS and MAXR selection with independent variables including all time points of TMP and pHi. Models with all variables significant at P < 0.10 were deemed valid.

Results: The pHi averaged 6.37 (6.03 to 6.85) and 20-h pH averaged 5.79 (5.43 to 6.02). The 40-min TMP averaged 38.8°C (32.7 to 40.8°C), 3-h TMP averaged 15.7C (5.6 to 25.1C), 6-h TMP averaged 8.1°C (2.4 to 15.5°C), and 20-h TMP averaged 2.4°C (0.0 to 4.4°C). Correlations between TMP and pHu from 40-min to 165-min and from 15-h to 19-h were not significant (P > 0.05; r = -0.03 to -0.30). Correlations between TMP and pHu from 180-min to 14-h were significant (P < 0.05; r = -0.35 to -0.51). Regression models for 1 (R<sup>2</sup> = 0.49), 2 (R<sup>2</sup> = 0.63), 5 (R<sup>2</sup> = 0.72), 6 (R<sup>2</sup> = 0.81), 7 (R<sup>2</sup> = 0.84), 8 (R<sup>2</sup> = 0.88), 9 ( $R^2 = 0.91$ ), 10 ( $R^2 = 0.92$ ), and 13 ( $R^2 = 0.95$ ) variable models (VARM) were generated for predicting pHu. All independent variables in the models were significant (P < 0.05) except in the 5, 6, and 13 VARM in which 1 or 2 of the independent variables tended to be significant (P < 0.10). As expected, the 1 VARM consisted of pHi and all subsequent models did as well. The pHi is critical as a baseline before the onset of chilling to account for peri-mortem stress levels and allow for a better prediction of pHu. The 2 VARM also included 45 min TMP and the 5 VARM added 50, 55, and 195 min TMP, with 195 min TMP remaining in subsequent models. Temperature at 45, 50, and 55 min were removed from the 6 VARM and replaced with 210, 225, and 345 min TMP and remaining in subsequent models. Temperature at 180 min was included in the 7 VARM but was not in subsequent models being replaced by 80 and 165 min TMP in the 8 VARM. Temperature at 165 min remained in all subsequent models and 80 min TMP was in all subsequent models except the 10 VARM. In the 9 VARM, 330 min TMP was added and remained in the 10 and 13 VARM. The 10 VARM added 100 min TMP and replaced 80 min with 90 min TMP. Temperatures at 90 and 100 were removed with 45, 75, 80, 95, and 360 min TMP being added.

**Conclusion:** These data indicate that loin TMP from 165 to 360 min PM is critical in the development of pHu. From a commercial perspective, these data can be used to attain desired pHu levels by precision management of the TMP decline curve.

Keywords: decline rate, pH, pork, quality, temperature



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COMPARISON OF LONGISSIMUS LUMBORUM AND PSOAS MAJOR METMYOGLOBIN REDUCING ABILITY AND OXYGEN CONSUMPTION QUANTIFICATION APPROACHES

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**Objectives:** Metmyoglobin reducing activity (MRA) and oxygen consumption (OC) are two important inherent biochemical properties that influence meat color. The American Meat Science Association Meat Color Guidelines describes various approaches to quantify MRA and OC. The objective of the current study was to assess and compare various combinations of MRA and OC incubation temperatures and meat surfaces related to meat color stability. Materials and Methods: Eight USDA Low Choice short loins were collected from a beef processor five days postmortem. The longissimus lumborum (LL; color stable) and psoas major (PM; color labile) muscles were separated from short loins and were sliced into 1.9-cm-thick steaks on day six postmortem. Steaks from both muscles were placed on foam trays with absorbent pads, wrapped with polyvinyl packaging film, and placed in a simulated retail display under continuous lighting. Strip steaks were evaluated on days 0, 3, 6, and 8, and tenderloin steaks were evaluated on days 0, 2, and 4. For MRA and OC on each color analysis day, steaks were cut into half and butterflied to create two surfaces – one surface exposed to air during display and the other a freshly-cut interior. Nitrite-induced metmyoglobin reduction was utilized for MRA analysis, while changes in oxymyoglobin levels of bloomed steaks following vacuum packaged were used to assess OC. For all analyses, the samples were incubated at 4°C or 37°C. Both MRA and OC were calculated as preincubation MRA, bloomed value, change of pre-incubation and post-incubation, and as a percentage. Hence, 12 combinations of MRA and OC values were used to assess their ability to determine these traits to follow display discoloration. Surface color readings were recorded using a HunterLab MiniScan spectrophotometer (2.5-cm diameter aperture, Illuminant A, and 10° standard observer). A split-plot design was used to assess the muscle-specific effects on MRA and OC. The data were analyzed using the PROC CORR and PROC REG options in SAS.

**Results:** As expected, PM discolored faster during display than the LL. For LL, change in metmyoglobin levels pre- and post-incubation of surface exposed to air incubated at 37°C had the best fit with  $a^*$  values (R<sup>2</sup> = 0.51). However, for PM pre-incubation metmyoglobin content of surface exposed to air during display incubated at 4°C had the best fit (R<sup>2</sup> = 0.41). For both LL and PM, bloomed values of surface exposed to air during display resulted in the best fit for OC (LL, R<sup>2</sup> = 0.76; PM, R<sup>2</sup> = 0.32). The best fit MRA and OC values for LL and PM also had the lowest coefficient of variation. The LL has more glycolytic fibers, and PM has oxidative (more mitochondria and myoglobin) fibers.

**Conclusion:** The LL has more glycolytic fibers, and PM has oxidative (more mitochondria and myoglobin) fibers. The current research suggests that the best fit for MRA and OC as measures of discoloration are muscle specific. Hence, using the same approach for two different muscle types may not provide accurate information about MRA and OC. Developing techniques to accurately predict color stability of meat during retail display is important to maximize the benefits of the post-harvest process to limit losses due to discoloration.

Keywords: Meat color, Beef, MRA, oxygen consumption



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#### THE EFFECT OF FATTY ACID SUPPLEMENTATION IN GESTATING EWES ON CARCASS CHARACTERISTICS AND MEAT QUALITY OF LAMBS FINISHED WITH DIFFERENT LEVELS OF FEED INTAKE

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**Objectives:** Maternal nutrition can alter the energy metabolism, muscle development, and body composition of offspring. Previous research suggested differences in the primary feed source of maternal winter-feeding diets during mid to late gestation caused differing fat and muscle deposition in finishing lambs. However, the effect of fatty acid supplementation in late gestation ewes on carcass characteristics and meat quality of their offspring has not been investigated yet. Therefore, the objective of this study was to examine the influence of fatty acid supplementation in ewes on lamb carcass characteristics and meat quality of longissimus lumborum (LL) muscle.

**Materials and Methods:** Pregnant ewes (n=24) were fed diets supplemented with 1% inclusion rate of fatty acid supplement for a 35-day period during the last trimester (day 100 to day 135) of gestation. Supplements were defined as either high in monounsaturated fatty acids (MUFA; 36.3% C18:1) or high in polyunsaturated fatty acids (PUFA; 9.18% C20:5 and 6.99% C22:6). At weaning, lambs were allocated in a 2 × 2 factorial treatment arrangement with maternal diet (MUFA or PUFA) and lamb feeding program (ad libitum or feed-restricted; 15% less than ad libitum) as main factors. Carcass data were collected and included hot carcass weight, body wall thickness, backfat thickness, LL muscle area, and an estimation of boneless trimmed retail cuts. Lamb LL (n = 24) from the right-side carcasses were collected approximately 72 hours postmortem and wet aged (2°C) in vacuum packages for an additional 4 days (total of 7 days postmortem aging). After the aging period, each LL muscle was fabricated into two 6.35-cm length sections, vacuum packaged, and randomly allotted to an internal endpoint cooking temperature of either 63°C or 71°C using a water bath before evaluated for instrumental color (Minolta spectrophotometer) and tenderness (TA-XT texture analyzer equipped with a Meullenet-Owen Razor Shear [MORS] fixture). The remaining section was used for uncooked instrumental color, muscle pH, and proximate composition (intramuscular fat [IMF%]) analysis. Carcass characteristic and meat quality results were analyzed using PROC MIXED of SAS, and differences among the means were performed using the least significant differences test at the 5% level.

**Results:** Hot carcass weight, body wall thickness, LL muscle area, estimation for boneless trimmed retail cuts, pH, instrumental color, tenderness, and IMF% were not influenced ( $P \ge 0.10$ ) by maternal fatty acid supplementation, finishing lamb feed intake, or their interaction (Table 1). Nonetheless, an interaction (P < 0.05) between maternal fatty acid supplementation and finishing lamb feed intake was observed for backfat thickness, with lambs from ewes with MUFA supplementation and assigned to restricted feeding and lambs from ewes with PUFA supplementation and assigned to restricted feeding. Image:



Parameter	Dietary treatment				P-value			
	MUFA		PUFA		-			
	Ad	Feed-	Ad	Feed-	Maternal	Finishing	Maternal diet x	
	libitum	restricted	libitum	restricted	diet	diet intake	Finishing diet intak	
Hot carcass weight, kg	26.95	24.60	25.44	24.53	0.46	0.14	0.51	
Backfat thickness, cm	0.40 <sup>ab</sup>	0.49 <sup>a</sup>	0.49 <sup>a</sup>	0.31 <sup>b</sup>	0.33	0.33	0.01	
Longissimus lumborum muscle area, cm <sup>2</sup>	17.53	15.05	16.13	16.56	0.96	0.34	0.18	
Bodywall thickness, cm	1.88	1.69	1.74	1.63	0.23	0.10	0.63	
Estimation for boneless trimmed retail cuts, %	48.26	47.88	48.07	48.86	0.30	0.59	0.13	
рН	5.61	5.66	5.66	5.65	0.64	0.54	0.49	
Intramuscular fat (IMF) content, %	2.85	2.60	3.23	3.07	0.17	0.49	0.89	
a* value (redness)-Raw	12.85	12.50	12.97	13.21	0.46	0.81	0.99	
a* value (redness)-63°C	10.37	10.01	9.49	9.86	0.23	0.99	0.38	
a* value (redness)-71°C	8.88	8.88	9.06	8.88	0.81	0.81	0.82	
MORS force-63°C, N	14.44	14.73	14.24	14.44	0.77	0.77	0.95	
MORS force-71°C, N	15.35	16.81	17.10	16.56	0.51	0.69	0.39	

Table 1. Effects of maternal fatty acid supplementation (MUFA or PUFA) and finishing lamb feed intake (ad libitum or feed-restricted) on carcass characteristics and meat quality of lamb longissimus lumborum muscle.

<sup>a,b</sup> Means lacking a common superscript letter within a raw are different (P < 0.05).

**Conclusion:** Overall, the observed interaction between maternal nutrition and finishing lamb feed intake altered backfat thickness, yet meat quality parameters were not influenced.

Keywords: carcass characteristics, fatty acid, fetal programing, lamb quality, maternal diet



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MODIFIED ALGORITHMS TO QUANTIFY SUB-SURFACE MYOGLOBIN FORMS USING NEAR-INFRARED DIFFUSE REFLECTANCE SPECTROSCOPY

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**Objectives:** The proportion of myoglobin forms on surface and sub-surface of meat dictates the visual appearance. Hence, quantification of myoglobin forms is of interest to researchers and industry. Techniques without sample preparation, rapid, and accurate are ideal considerations while designing methods to determine myoglobin forms. Reflectance spectrophotometry, the current standard of color evaluation in meat, assesses metmyoglobin near the surface. By configuring the illumination and collection of broad-band light sensitive to myoglobin absorption to probe deeper meat, sub-surface myoglobin formation can be measured. Commercially available near-infrared (NIR) based spectrophotometry techniques are designed to quantify oxy- and deoxymyoglobin as metmyoglobin content is physiologically negligible. Here we discuss a novel algorithm to quantify metmyoglobin content using NIR-based diffuse reflectance spectroscopy (DSR).

**Materials and Methods:** Beef *longissimus lumborum* muscles were collected from a commercial processor day five postmortem (n = 8 replications). Steaks were cut, packaged in polyvinyl chloride film, and displayed under simulated retail display cases (Philips Neutral White LED lamps; Andover, MA; color rendering index = 82; color temperature = 3,000° K) for eight days. Oxymyoglobin (OxyMb), deoxymyoglobin (DeoxyMb), and metmyoglobin (MetMb) content on beef *longissimus lumborum* muscles were determined daily for eight days using a HunterLab MiniScan spectrophotometer and in-house developed NIR-DRS. Daily changes in subsurface myoglobin redox states at a probing depth of approximately 1.5 mm were evaluated using NIR-DRS and compared with surface color assessed by HunterLab MiniScan spectrophotometer (a 2.5-cm diameter aperture, illuminant A, and 10° standard observer). A custom mathematical model programmed in MATLAB was used to assess deoxy-, oxy-, and metmyoglobin. The model utilized approximately eighteen wavelengths spanning from 480-650 nm to quantify myoglobin forms.

**Results:** Both HunterLab MiniScan and NIR-DRS measurements revealed that MetMb increased steadily over the duration of display, and both demonstrated a high correlation ( $R^2 = 0.91$ ) between the two methods. NIR-DRS revealed the OxyMb to have decreased steadily over the period of display. However, HunterLab MiniScan spectrophotometer indicated a much later onset of the apparent decrease of OxyMb than NIR-DRS reading, resulting in a moderate correlation ( $R^2 = 0.64$ ) between the two methods. No correlation was found between the two methods regarding the changes of DeoxyMb over the duration of the display.

**Conclusion:** By probing deeper muscle using myoglobin-sensitive spectrum - than the conventional reflectance spectrophotometty, the newly developed NIR-DRS approach has potential as an alternative method for the color assessment in post-rigor skeletal muscle.

Keywords: Diffuse reflectance spectroscopy, Meat color, Myoglobin, NIR, Reflecto-spectrophotometry



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#### COMPARISON OF MYOGLOBIN QUANTIFICATION METHODS

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**Objectives:** Quantification of meat color changes is important to understand discoloration. Various approaches are reported in the literature to quantify myoglobin forms using both destructive and non-destructive approaches. In the sarcoplasmic extraction procedure, samples at specific days of storage are homogenized in buffer, and the supernatant is used to determine myoglobin forms. In Krzywick's method, reflectance values are converted to absorbance and inserted in appropriate equations to determine myoglobin forms. The American Meat Science Association Meat Color Guidelines describes the use of K/S ratios at isobestic points to quantify myoglobin forms. K/S ratio takes into account absorption (K) and scattering (S) components of meat. However, limited studies have compared different methods to quantify deoxymyoglobin (DeoxyMb), oxymyoglobin (OxyMb), and metmyoglobin (MetMb). The objective of the study was to compare three myoglobin quantification methods – sarcoplasmic extraction, Krzywick's method, and K/S methodology.

**Materials and Methods:** Six USDA Select beef strip loins from a commercial processing plant were cut into 1.9 cm thick steaks, placed onto foam trays with absorbent pads, and over-wrapped with a polyvinyl chloride film. The steaks were displayed on a simulated retail display case for eight days. Three steaks from each loin were also utilized to prepare 100% standards of DeoxyMb, OxyMb, and MetMb using the K/S method. Myoglobin forms were determined using three methods every 48 h of display. The data were analyzed using regression, correlation, and coefficient of variation options in SAS. The data for each myoglobin form was analyzed separately.

**Results:** Krzywicki's method had a lower coefficient variation (P < 0.05) for OxyMb compared with the sarcoplasmic and K/S methods. However, the range of OxyMb values for Krzywick's method was narrow compared with sarcoplasmic and K/S methods. The coefficient of variation for the sarcoplasmic method was greater (P < 0.05) for MetMb compared with the other two methods. Tissue homogenization steps in sarcoplasmic method can promote more oxidative changes. The coefficient of variation was greater (P < 0.05) when sarcoplasmic and K/S methods were used to quantify DeoxyMb. **Conclusion:** In general, the K/S method captured a great range of OxyMb and MetMb changes than did Krzywick's and sarcoplasmic extraction methods. Developing techniques to accurately measure myoglobin forms are important to understand meat color changes.

Keywords: Meat color, Myoglobin, Sarcoplasm



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CHARACTERIZING CARCASS CONFORMATION, QUALITY ATTRIBUTES AND MUSCLE FIBER PROPERTIES OF BEEF × DAIRY CROSSBRED CATTLE

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**Objectives:** The increase in number of crossbred cattle resulting from the mating of conventional beef-type bulls and dairy cows has elevated the need for research pertaining to beef resulting from this scenario. The objective of this study was to characterize this changing segment of the U.S. fed-beef supply on the basis of meat quality and immunohistochemical properties of beef resulting from beef × dairy (BD) crossbred cattle relative to conventional beef (CB) and dairy type (DT) cattle.

**Materials and Methods:** At 3 different commercial beef packing facilities, BD, CB and DT carcasses (N = 560) were selected to equally represent USDA Prime, Upper 2/3 Choice, Choice and Select quality grades. Following carcass selection, strip loin sections (5 cm thick) were collected from both carcass sides and stored at (2 to 4°C) in the dark for 14 days. At the same time, samples were collected from the *longissimus lumborum* were collected for immunohistochemical analysis (n = 113). The immunohistochemical samples were cut parallel to muscle fibers and immediately embedded in clear section compound and frozen using dry ice and 2-methyl-butane and stored at -80°C. Muscle fiber cross-sectional area and myosin heavy chain (MHC) isoform were determined by cutting 10µm-thick cross-sections perpendicular to the muscle fibers at -19°C. Aged strip loin sections designated for quality analysis were fabricated into 4, 2.54 cm steaks, vacuum packaged and stored at -20°C. At the time of fabrication, steaks were randomly assigned to shear force, trained sensory, consumer sensory, or retail display. Only samples from USDA Choice carcasses were evaluated for retail display and color performance. Data were analyzed as a completely randomized experiment with a 3×4 factorial arrangement. Cattle type, USDA Quality Grade and their interaction (when appropriate) were included as fixed effects.

**Results:** All evaluated carcass traits were impacted by cattle type (P < 0.01). BD carcasses were intermediate to CB and DT in fat thickness, REA, and carcass length. *Longissimus* samples from BD carcasses had the greatest proportion of myosin heavy chain (MHC)-IIA fibers ( $P \le 0.01$ ) and the largest mean cross-sectional area ( $P \le 0.05$ ). Cattle type impacted pH, trained color panel attributes, L\* and a\* values and shear force measurements (P < 0.01). Trained sensory analysis indicated that tenderness, juiciness, sour, metallic, fat-like, buttery, liver and oxidized attributes were affected by cattle type (P < 0.01). Consumers identified a difference in tenderness among cattle types (P < 0.01). **Conclusion:** Crossbreeding beef sires and dairy cows will continue to change the dairy influenced portion of the U.S. fed beef supply. Beef from crossbred beef x dairy cattle can be expected to perform with superiority in flavor and tenderness compared to beef from conventional beef breeds, without shelf-life concerns at retail display associated from beef from Holsteins. Marketing programs founded on eating quality should consider inclusion of beef from beef x dairy crossbreds.

Keywords: beef, color, dairy, fiber type, sensory



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MEASURING THE REPEATABILITY AND ACCURACY OF RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY (REIMS) USING PORK AND MULTIPLE PREDICTION TECHNIQUES

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**Objectives:** Rapid evaporative ionization mass spectrometry (REIMS) has shown potential in the meat and food industries to identify food fraud, classify samples and potentially predict quality attributes. While recent research has shown REIMS to be a capable method of sample differentiation, the reliability and repeatability of the machine has yet to be fully examined. In this study, the repeatability and prediction accuracy of REIMS for identifying homogenized pork loin (N = 360), technician (n = 3), and day of analysis (n = 3), was explored.

**Materials and Methods:** The M. longissimus thoracis et lumborum (LTL) muscle was isolated from three boneless pork loins purchased from a local club store on the same day, and efforts were made to select pork loins originated from different animals, based upon visual assessments. For each pork loin, the LTL was completely denuded, flash frozen in liquid nitrogen, pulverized/homogenized. Five-g aliquots of the homogenous mixture were placed into multi-well trays (12 wells each) in a random order. Samples were kept frozen (-80° C) until further analysis. Trays were randomly and equally distributed across technician (n = 3) and day of analysis (n = 3). Homogenate mixtures representing the individual pork loins were analyzed for proximate analysis. Each day, samples were thawed in a refrigerator prior to being analyzed (12-18 hours), and spectra were acquired from the individual samples using a Synapt G2-OSi Q-TOF equipped with REIMS and an iKnife sampling device in negative resolution mode. This study was analyzed as a completely randomized design where in a repeated measure analysis of variance (ANOVA) day and technician served as fixed effects and tray served as a random effect. Principal component analysis (PCA) was used as an explorative technique for determination of metabolomic differences between pork loins. Lastly, a multivariate approach, Quadratic Discriminant Analysis (QDA), was used for determining classification of pork loin ID, technician, and REIMS day. **Results:** Proximate analysis indicated no significant differences in composition between pork loins (P > 0.05); however, using QDA and the data acquired from the REIMS spectra, the model was able to identify the correct pork loin with a 71.67% accuracy. Additionally, a QDA model was able to identify the day of sample acquisition with an 80.8% accuracy, indicating a day effect in the data collection procedures. Conversely, the QDA model was able to correctly identify the technician 36% of the time indicating that operator and/or technician had a minimal effect on data acquisition over the 3 days of analysis. When spectra were analyzed using a PCA, peaks at 279.25 m/z (linoleic acid) and 281.25 m/z (oleic acid) were determined to be the predominant drivers of variability between individual loin samples. **Conclusion:** In conclusion, REIMS displayed its ability to accurately separate highly similar samples regardless of technician. However, an unidentified day effect could potentially affect results over an extended testing interval. Further research into the contributors to the day effect, as well as the machine's tolerance to this effect is warranted.

Keywords: Machine learning, Mass spectrometry, Metabolomics, Pork, REIMS



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DIVERGENT PHENOTYPES OF CROSSBRED BEEF X DAIRY CATTLE MINIMALLY INFLUENCE EATING QUALITY AND CARCASS PERFORMANCE

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**Objectives:** Claims of considerable visual variation in crossbred beef × dairy cattle, combined with negative associations of dairy influence, have produced questions about their inclusion into quality-driven branded programs. Varying expressions of visual phenotype in crossbred cattle with equal *Bos indicus* influence has previously impacted eating quality. The present study evaluated effects of visual phenotype expression in crossbred beef × dairy cattle on carcass performance and eating quality.

**Materials and Methods:** A panel of 3 expert evaluators assessed a total of 615 crossbred (Angus or Simmental × Angus bulls mated to Holstein cows) beef × dairy calves from 9 commercial feedlot pens for muscling and frame size. Visual assessments were used to subset each pen and categorize cattle into 4 phenotype groups (n = 82 to 84 cattle per group). Carcass data were collected, and strip loin steaks from each animal were evaluated for dimensionality, shear force, trained sensory performance (tenderness, juiciness, and flavor notes), retail color display, fatty acid composition, and myosin heavy chain isoforms. Data analyses tested for fixed effect of phenotype group and accounted for random effect of pen (block).

Item	Dairy-like		Beef-like		SEM <sup>2</sup>	P-value
	Group 1	Group 2	Group 3	Group 4		
Live traits						
Muscling score <sup>3</sup>	2.8 <sup>d</sup>	4.0 <sup>c</sup>	4.5 <sup>b</sup>	5.6ª	0.18	< 0.01
Frame size score <sup>3</sup>	3.1 <sup>d</sup>	4.4 <sup>c</sup>	5.9 <sup>b</sup>	7.1ª	0.16	< 0.01
Carcass traits						
Round muscling conformation <sup>3</sup>	3.8 <sup>c</sup>	4.4 <sup>b</sup>	4.8 <sup>b</sup>	5.3ª	0.17	< 0.01
Carcass length, cm	154ª	152 <sup>b</sup>	150 <sup>c</sup>	149 <sup>c</sup>	0.8	< 0.01
Myosin heavy chain (MHC) isoforms						
MHC-I, %	20.3	23.1	21.7	23.4	1.26	0.26
MHC-IIA, %	34.4ª	33.2 <sup>ab</sup>	28.2 <sup>b</sup>	31.6 <sup>ab</sup>	2.74	0.03
MHC-IIX, %	45.3	43.7	50.1	45.0	2.97	0.08

<sup>1</sup>Group 1 cattle were selected as most dairy-like in appearance, and Group 4 cattle were selected as most beef-like in appearance.

<sup>2</sup>Standard error of the means (SEM), pooled.

<sup>3</sup>Scores: 1 = light muscled, large framed; 9 = heavy muscled, small framed.

<sup>a-d</sup>Estimated marginal means with different superscripts differ (P < 0.05).

**Results:** Although visual appearance (muscling and frame size) – by design – was distinctly different (P < 0.05) among phenotype groups of beef × dairy crossbreds evaluated in this study, few – if any – differences (P < 0.05) were noted in



trained sensory performance, shear force, steak dimensionality, fatty acid composition, and retail color display. Crossbreds that were more dairy-like in appearance exhibited a lesser (P < 0.05) proportion of glycolytic muscle fibers, suggesting opportunity for more aggressive use of growth technologies, without detriment to eating quality. Beef-like crossbreds exhibited shorter (P < 0.05) carcasses with greater (P < 0.05) round muscling conformation than dairy-like crossbreds – even when carcass weights and ribeye areas did not differ ( $P \ge 0.28$ ) – indicating a shift in distribution of muscularity.

**Conclusion:** Live expression of dairy or beef character in crossbred beef × dairy cattle minimally contributed to differences in carcass performance or eating quality. Additional research is needed to understand carcass yield and muscle fiber type composition of beef × dairy crossbreds. Nonetheless, efforts to exploit complementarity of beef genetics in terminal beef × dairy systems cannot be expected to negate consistency in eating quality of resulting product.

Keywords: beef, dairy, eating quality, fiber type, phenotype



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#### OBSERVED AND DERIVED MEASUREMENTS OF BEEF CARCASS CHILLING RATE: THEIR RELATION TO EACH OTHER AND CARCASS TRAITS

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**Objectives:** The objectives of this research were to evaluate methods of reporting chilling rates of beef carcasses, including observed and derived measurements, and how these measurements relate to each other and carcass traits. The knowledge obtained from the above objectives may indicate what measures of chilling rates are best used when evaluating beef carcass chilling.

**Materials and Methods:** Beef carcasses (n = 53) representing various hot carcass weights were randomly selected over 5 nonconsecutive days at a commercial beef processing facility after harvest and immediately before entering the chill cooler. Carcasses were outfitted with a data logger which recorded temperature every 15 minutes for 24 hours. One temperature probe was inserted into the *longissimus thoracis* (LT) at approximately the 6<sup>th</sup> rib and the other temperature probe was inserted into the direct center of the *semimembranosus* (SM). After a 24 h chill, carcass data including, hot carcass weight, 12<sup>th</sup> rib fat thickness, ribeye area, kidney, pelvic, and heart fat percentage, and marbling score were collected by trained personnel. After chill data retrieval, derived measures of chilling rate were calculated, including determining the total area under the chilling curve (AUC) for the 0-6 h and 0-24 h chill time windows via the trapezoidal method with temperature as the y-axis and time as the x-axis. Additionally, the average slope of the temperature decline curve was calculated. Pearson correlations coefficients among carcass temperatures at various times (0, 4, 8, 12, 16, 20, and 24 hrs), derived measures of chilling rate, and carcass traits were found using the PROC CORR procedure of SAS (Version 9.4, SAS Institute, Cary, NC).

**Results:** In both the LT and SM, derived slope of the temperature decline curve was not significantly correlated with other temperature measures ( $r \le 0.42$ ,  $P \ge 0.08$ ). In the LT and SM, 8 h and 12 h time points were significantly correlated with other temperatures (0, 4, 6, 8, 12, 16, 20, 24 h, AUC 6 h, and AUC 24 h) ( $r \ge 0.54$ ,  $P \le .0001$ ). In the LT, 20 and 24 h temperature measures were significantly correlated to 12<sup>th</sup> rib back fat thickness (r = 0.50, P = 0.0001, r = 0.52, P = <.0001, respectively). Conversely, in the SM, 12, 16, 20, and 24 h temperature measures were significantly correlated to hot carcass weight ( $r \ge 0.37$ ,  $P \le 0.0089$ ).

**Conclusion:** Our results indicate using raw chilling rate data, as well derived AUC measures are appropriate methods to evaluate beef carcass chilling rates. Additionally, our results indicate the 8 and 12 h time points have the highest correlations with other temperature decline data, however, other time points were also significantly correlated and could be used. Furthermore, our results indicate for the LT, 12<sup>th</sup> rib fat thickness is more highly correlated to temperature decline than other carcass measurements. Conversely, for the SM, hot carcass weight is more highly correlated to continue to understand how best to evaluate beef carcass chilling rate data, as well as how various carcass measurements may affect overall beef carcass chilling rates.

Keywords: beef chill, carcass characteristics



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QUALITY AND CARCASS CHARACTERISTICS OF MEAT RABBITS FED HEMPSEED MEAL

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**Objectives:** Industrial hemp has been used in the U.S. to make fiber from stalks, food from hempseed, and oil from flowers. After oil extraction, hempseed meal (HSM) is a high protein byproduct that can potentially be used as a livestock feed source. The objective of this study was to evaluate the carcass characteristics of meat rabbits supplemented with HSM as a protein replacement in the ration.

Materials and Methods: Four rations were formulated and fed to rabbits containing varying inclusion rates of HSM: 0% (Control; n = 8), 25% (n = 8), 50% (n = 8), and 75% (n = 7) replacement of protein source in a pelleted feed. Rabbits were fed for 35 d and harvested at Tarleton State University Rabbitry following IACUC guidelines (AUP 02-002-2021). Live weight was taken 12 h prior to slaughter and hot carcass weights (HCW) were collected immediately after slaughter. Carcass measurements were taken while suspended by the Achilles on leg hooks measuring 20 cm wide. Length of leg was measured from the calcaneal tuberosity to the posterior edge of the ilium. Length of body was measured from the posterior edge of the ilium to the anterior edge of the first rib. Length of loin was measured from the anterior edge of the ilium to the posterior edge of the last rib. Width of leg and width of shoulder was measured from the ventral edge of the flank and elbow pockets, respectively. Muscle pH was taken 0- and 24-h postmortem in the leg, loin and shoulder. Rabbits were fabricated and individual weights of lean, fat and bone were collected. Percent carcass composition was determined by dividing the lean, fat and bone by the HCW. Data were analyzed as a generalized complete block design using the GLIMMIX model in SAS v. 9.4 (SAS Institute, Cary, NC, USA). Statistical significance was set at  $P \le 0.05$ . **Results:** Rabbits fed 75% HSM had a lower (*P* < 0.05) final live and HCW compared to control, 25% and 50% HSM. Rabbits fed 75% HSM had shorter carcasses (P < 0.05) compared to all other treatments. Rabbits supplemented with 50% HSM had a wider leg (P < 0.05) compared to 75% HSM. Also, there was no difference (P > 0.05) in leg width between the control, 25% and 50% HSM. Rabbits fed the control had a longer loin (P < 0.05) compared to 75% HSM. Additionally, there was no difference (P > 0.05) in loin length between control, 25% and 50% HSM. There was no difference (P > 0.05) for the length of leg and width of shoulder between treatments. At 0 h postmortem, there was no difference (P > 0.05) in muscle pH between treatments. However, rabbits fed 75% HSM had the highest (P < 0.05) pH 24 h postmortem. Lastly, there was no difference (P > 0.05) between treatments in percent lean, fat, or bone in the carcass composition analysis.

**Conclusion:** Rabbits fed HSM at 25% or 50% of the protein source resulted in no changes in carcass length, composition, and quality as compared to the control and may be an advantageous supplement to the livestock industry. However, rabbits fed a 75% HSM ration resulted in lighter live and carcass weights while producing a shorter carcass with shorter loins, narrower legs, and a higher overall pH 24 h postmortem and should not be considered as a replacement level.

Keywords: Carcass Composition, Hempseed Meal, Quality, Rabbit



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USING RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY (REIMS) AS A NOVEL, MINIMALLY INVASIVE, REAL TIME METHOD FOR CHARACTERIZATION OF METABOLIC VARIATION CONTRIBUTING TO FLAVOR, TENDERNESS, AND COLOR STABILITY OF BEEF

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**Objectives:** Rapid evaporative ionization mass spectrometry (REIMS) is relatively new technology that has emerged in both human and food science. REIMS, a new method of collecting data from intact biological tissue, allows for little to no sample preparation and very fast sampling times. Online, rapid detection of metabolites that contribute to differences responsible for meat quality attributes has major implications. This study evaluated the ability of REIMS to classify beef based on palatability and color stability.

**Materials and Methods:** Samples were obtained from a previous study in which USDA Choice strip loin steaks (N = 191) were selected for variation in predicted slice shear force, objective color measurements, and trained sensory evaluation of flavor. Metabolomic profiles (50 to 1200 m/z) were generated by REIMS in negative resolution mode. Individual samples were classified into performance groups of palatability and color stability using principal component analysis and k-means clustering. Discriminant and quadratic function analyses classifications evaluated the accuracy of the predictive model.

**Results:** REIMS correctly characterized 96.34% of samples sorted on objective color measurements, using 2 discriminant functions explaining 97.44% in discriminant function 1 (P < 0.001) and 2.45% in discriminant function 2 (P < 0.001) of variation in the data set. Performance groups were distinctly different in objective color measurements ( $P \le 0.01$ ). However, samples had limitations in variability of tenderness and sensory attributes, restricting the capability of REIMS to differentiate between specified performance groups at a relatively low accuracy of 47.64%.

**Conclusion:** Due to the variation in color and color stability, REIMS had the ability to identify color differences with a high level of accuracy. Additionally, with greater variability and a larger sample size, REIMS shows significant promise in detecting flavor and tenderness differences as well as color stability performance groups of beef.

Keywords: beef, machine learning, meat quality, REIMS, sensory



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PRELIMINARY ASSESMENT OF THE MEAT IMAGING JAPAN (MIJ) BEEF GRADING SMART PHONE APPLICATION AS IT COMPARES TO A CURRENT USDA VALIDATED BEEF GRADING CAMERA VISION SYSTEM

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**Objectives:** Camera vision beef grading systems can be cost prohibitive to smaller beef processers which results in limited advanced technology grading capabilities. The objective of this study was to compare an alternative beef carcass grading technology with a commercially available USDA approved beef grading camera (VBG2000, E+V Technology). **Materials and Methods:** Beef carcasses (n = 910), representing marbling standards from USDA Select, Choice, and Prime in youthful, commercially raised beef cattle, served as the experimental units. All beef carcasses utilized were evaluated in a large-scale commercial beef harvesting facility and assessed on-line in the grading cooler. Data were collected using the MIJ beef grading application software and camera cradle accessory lighting kit with a Google Pixel 4 XL platform. The MIJ camera data was collected in unison with that of a commercially available, USDA approved, on-line instrument grading technology (VBG2000, E+V Technology GmbH & Co. KG, Oranienburg, Germany). Carcass grading information was analyzed using Pearson Correlation analyses.

**Results:** The marbling scores obtained by the MIJ camera observations had a strong correlation to the currently validated beef grading vision system (r = 0.71; P < 0.01). The ribeye areas of the evaluated carcasses ranged from 61.87 cm<sup>2</sup> to 127.55 cm<sup>2</sup>. The ribeye areas observed by the MIJ camera were moderately correlated to the currently validated beef grading vision system. (r = 0.56; P < 0.01).

**Conclusion:** The initial validation of an alternative beef grading system suggests an opportunity to pursue additional validation for further use in USDA processing facilities. Ultimate utilization of the MIJ camera vision system will allow for more beef producers and processors to capture objective beef carcass data across a wider range of processing facilities.

Keywords: beef, camera grading, ribeye size



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NOVEL NEAR-INFRARED BASED NEEDLE PROBE TO ASSESS INTERIOR MEAT COLOR

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Objectives: As consumers prefer a bright cherry-red color of steaks, discoloration leads to less consumer acceptance. Metmyoglobin formation results in discoloration, and this process starts at the interior of steaks and spreads to the surface. Researchers utilize surface color measurements to study meat color chemistry. Limited surface-based studies have evaluated interior color changes to understand meat discoloration. Here, we discuss a novel near-infrared (NIR) based probe to study localized interior meat color. The objective was to use a needle-probe enabled NIR diffuse reflectance spectroscopy (DRS) to evaluate localized interior color changes in *psoas major* steaks during retail display. **Materials and Methods:** Six *psoas major* steaks from different loins were collected from a regional processing facility and transported on ice to Oklahoma State University. The steaks were cut, packaged in PVC overwrap, and placed in a simulated retail display for five days. During retail display, surface color was evaluated every day in triplicate using HunterLab MiniScan Spectrophotometer to quantify changes in surface color of the steaks. Subsurface localized myoglobin forms from 1 mm to 6 mm depths. In this study, myoglobin forms were evaluated daily at 1 mm intervals using the needle-probe NIR-DRS. The needle-probe consisted of two 320 µm fibers epoxied side-by-side for strainconfined positioning within a 17-gauge spinal needle. An applicator was developed to place the needle-probe at 6 positions spaced laterally at an interval of 10 mm and inserted at 6 depths at increments of 1 mm. A custom mathematical model programmed in MATLAB was used to assess deoxy-, oxy-, and metmyoglobin from the needleprobe data. A HunterLab MiniScan spectrophotometer was also used to record surface  $L^*a^*b^*$ . Surface color data measured by HunterLab MiniScan spectrophotometer were analyzed using PROC Mixed Procedure of SAS. **Results:** As retail time increased, the steaks were darker (decreased in L\* values) and less red (decrease in a\* and chroma values, P < 0.05). The NIR-DRS needle-probe revealed differences in myoglobin form at various depths within each steak and during retail display for each steak at the same depth. The needle-probe revealed that all steaks at day-0 had greater deoxymyoglobin content at approximately 3 mm (75.64%) than the shallower layer (62.14%), which was consistent with lesser oxygen to bind with myoglobin at greater depth than at the surface. The needle probe also revealed that oxymyoglobin content below 2 mm depth increased throughout storage time in all steaks. The results indicated deeper penetration of oxygenation over time. Metmyoglobin formation increased at all depths during retail time. At 6 mm, metmyoglobin was at 0.19% at day 0 and increased steadily to 9.21% by day 4 of retail display. A similar trend in metmyoglobin formation was reported in the shallower layers paralleling with the decline in a\* values through retail display.

**Conclusion:** In conclusion, an in-house developed NIR-DRS needle-probe and algorithm provided information about localized internal color changes in post-rigor skeletal muscle. Localized characterization of internal color changes helps to understand muscle-specific, packaging, and aging-related color changes. In addition, determining interior metmyoglobin content helps to predict discoloration before visually seen on surface.

Keywords: Diffuse reflectance spectroscopy, Meat color, Myoglobin, NIR, Reflecto-spectrophotometry



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EVALUATING THE ABILITY OF RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY (REIMS) TO QUANTIFY COMPOUNDS RELATED TO BEEF QUALITY

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**Objectives:** Though gas chromatography (GC) methods have long served as the standard for quantifying compositional elements in meat products, they require extensive sample preparation, are very costly, and do not produce real-time results. Conversely, rapid evaporative ionization mass spectrometry (REIMS) produces metabolomic profiles comparable to GC methods in real-time and does not require sample preparation. This study evaluated the ability of REIMS to characterize compounds related to beef quality at time of grading.

**Materials and Methods:** Strip loin steaks were collected at the time of grading (4 d post-mortem) from A maturity carcasses of classifications: USDA Prime, Upper 2/3 Choice, Low Choice, USDA Select, Wagyu, Grassfed, and Dark Cutter. Metabolomic profiles measured by REIMS in negative sensitivity mode on intact steaks were compared to compositional profiles – amino acids, fatty acids, and volatile compounds – measured by conventional GC methods on steak homogenates. Using discriminant function analysis (DFA), metabolomic profiles from REIMS and GC methods were separately evaluated to determine their accuracy in characterizing samples into carcass classifications. Partial least squares (PLS) regression models using trained-test validation evaluated the ability of REIMS to predict amounts of 100 different compounds measured by GC.

**Results:** A DFA demonstrated comparable ability of REIMS to achieve a similar result as GC for determining carcass classification (as mentioned above) with 94% and 90% variation explained and 33% vs. 43% prediction accuracy, respectively. However, a PLS analysis using REIMS spectra (50 - 1,500 m/z) from a 4 d sample was unsuccessful in predicting quantities of individual compounds, in reference to the same compounds quantified by GC methods ( $R^2 \le 0.27$ ).

**Conclusion:** There is significant work to be done for the development of REIMS procedures to identify and quantify individual compounds in tissue samples, which includes new data preprocessing methods and more targeted variable selection for models, as well as implementation of internal standards and consultation of a mass spectrometry library.

Keywords: beef, Gas chromatography, Mass spectrometry, quality, REIMS



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#### REAL-TIME PREDICTION OF PORK LOIN QUALITY AND PALATABILITY BY A METABOLOMIC PROFILE COLLECTED BY RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY

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**Objectives:** It has been suggested the pork industry implement a quality grading system, using factors like marbling and color score, to sort pork carcasses into meaningful groups like palatability ratings [HT1]. Rapid evaporative ionization mass spectrometry (REIMS) has shown promise to accurately predict sensory attributes and quality categories of pork chops using two prediction model techniques, principal component linear discriminant analysis (PC-LDA) and linear discriminant analysis with an F-test applied (F-test-LDA).

**Materials and Methods:** Duroc × White line crossbred barrows (n=203) and gilts (n=197), representing different sire lines, were harvested at the same facility 3 consecutive weeks, at approximately 6 months of age and paired boneless center-cut pork loins (N=400) were collected. South Dakota State University collected yield measurements using the BioQ Scanner and reported a numerical color score (Pork Color Standards, National Pork Board, 1999), marbling score, Minolta L\*, a\* and b\*, pH, and Warner-Bratzler shear force values. Metabolomic profiles were obtained from a raw, intact pork chop using a Synapt G2 Si Q-TOF, coupled with a REIMS ionization source and an iKnife sampling device in negative resolution mode. A paired pork chop was evaluated by trained sensory panelists for tenderness, juiciness, and flavor attributes. Each sensory or quality attribute was transformed from a continuous value to a categorical response-low, medium, high – based on distance from the respective mean. Then, PC-LDA and F-test-LDA models used REIMS to classify individual samples into low, medium, and high categories.

**Results:** F-test-LDA models presented accuracy classification percentages of greater than 95% for marbling scores, color scores, and tenderness. Additionally, sensory attributes were classified correctly with high accuracy: pork flavor intensity - 97%, boar taint - 89%, oxidized - 86%, and other sensory flavors - >94% of samples.

**Conclusion:** These modeling techniques coupled with REIMS show promise as part of the implementation of a pork quality grading system. Further research of REIMS metabolomic profiles, along with predictive models is warranted for understanding REIMS full capabilities in predicting palatability.

**Keywords**: Metabolomics, Pork Grading System, Pork Palatability, Pork Quality, Rapid Evaporative Ionization Mass Spectrometry (REIMS)



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#### EFFECTS OF DRY AND WET-AGING ON SENSORY ATTRIBUTES, TENDERNESS, AND VOLATILE PROFILE OF USDA SELECT STRIP LOINS.

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**Objectives:** Evaluate the effects of dry and wet aging on sensory attributes, WBSF, and volatile profile of USDA Select short loins aged 21 and 80 days.

Materials and Methods: Thirty-two USDA Select short loins were arranged to a 2x2 factorial to evaluate the effects of aging method (dry and wet), length (21 and 80 d post-mortem) and their interaction. Samples were dry-aged at 2°C ±2 with 80-85% of relative humidity and air speed of 2 m/sec. Wet-aged samples were stored under vacuum at same temperature. After aged, strip steaks were fabricated, and sensory analysis was conducted with 8 panelists. Tenderloins were tested separately. For volatiles, steaks were analyzed in three zones, dorsal, intermediate, and ventral portions (2x2x3 factorial). Volatiles were captured using SPME fibers that were desorbed on a GCMS (Shimadzu Co., Kyoto, Japan). A texture analyzer was used to determine WBSF (Food Technology Co., Sterling, VA, U.S). Data were analyzed as a CRD using PROC GLIMMIX, PROC FREQ and PROC CORR of SAS. The level of significance was P≤0.05. **Results:** No effects of aging length or method were observed on WBSF, tenderness, juiciness and connective tissue. Panelists scored samples aged 80 d with higher off-flavor intensity when compared to samples aged for 21 d. Within 80 days of aging, wet-aged loins had higher off-flavor intensity than dry-aged. Steaks from loins aged 21 days were more desirable than steaks from loins aged for 80 days. Panelists scored higher frequencies of liver off-flavor in wet-aged samples and higher frequencies of sour and bitter off-flavors in loins aged for 80 days. Aging beef for 80 days led to higher concentrations of octanal, nonanal, decanal, and 2-5/6-Dimethyl pyrazine and lower concentrations of pentanal and acetoin. Dry aging led to higher concentrations of hexanal, heptanal, octanal, 3-Methylbutanal, 2-Pentylfuran whereas wet aging increased concentrations of Trimethyl pyrazine. When looking at steak zones, dorsal portions (C) of steaks dry aged for 21 days showed higher concentrations of hexanal, heptanal, octanal, and 2-Pentylfuran. Ventral portions of steaks wet aged for 21 days showed higher acetoin concentrations. Regarding effects of volatiles on offflavor intensity and overall desirability, dimethyl disulfide was positively correlated to off-flavor intensity and negatively correlated to overall desirability in steaks either wet aged or aged for 80 days. Pentanal was also negatively correlated to overall desirability in steaks aged for 80 days. In dry-aged steaks, 3-methylbutanal and 2-butanone were positively correlated with overall desirability. Positive correlations between overall desirability and decanal and trimethylpirazine were also observed in steaks aged for 21 days.

**Conclusion:** Aging Select short loins either for 21 and 80 days or using wet or dry methods do not affect juiciness and tenderness. Long aging periods may lead to higher off-flavor intensity, especially livery in wet aged and sour and bitter in dry-aged beef. Three methylbutanal and 2-butanone are desirable volatiles from a desirability standpoint whereas the first one is more abundant in dry-aged beef. Dry aging for periods about 21 days may improve sensory perception of USDA Select beef.

Keywords: beef, dry-aging, meat quality



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INFLUENCE OF SIRE BREED OF BEEF × HOLSTEIN CATTLE ON CARCASS TRAITS, MUSCLE FIBER PROPERTIES AND EATING QUALITY

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**Objectives:** Angus and Continental-influenced breeds are the most prevalent sire breeds utilized in the U.S. beef × dairy crossbreeding model; thus, in this study, beef quality differences among crossbred cattle types were evaluated and compared to fed-Holstein cattle.

**Materials and Methods:** Carcasses of crossbred beef × dairy cattle (breed verified by genotype) from either Angus (n=88), Continental breed influenced (n=85), or Holstein (n=118) sires and Holstein dams were selected to equally represent USDA Prime, Upper 2/3 Choice, commodity Choice and Select grades. Anterior sections of strip loins were collected from both sides of each carcass. A subset of samples (N = 67) from the *longissimus lumborum* were collected and frozen for myosin heavy chain (MHC) isoform determination. Strip loin sections were fabricated into steaks (2.54 cm thick) and were designated to analyses: shear force, trained sensory, consumer sensory, and retail display. Only steaks from USDA commodity Choice carcasses were displayed at retail for 96 hours. Steaks were imaged for steak dimensionality and pH was measured. Data were analyzed as a completely randomized design in a 3×4 factorial arrangement of breed type and quality grade. Breed type, USDA quality grade, and their interaction were included as fixed effects.

**Results:** Carcasses from cattle sired by continental influenced breeds were intermediate in 12<sup>th</sup> rib fat thickness (P < 0.01) to carcasses from cattle sired by Angus and Holstein. Continental sired beef × dairy crossbreds possessed the largest ribeye area and had the lowest numerical yield grade (P < 0.01). Straightbred Holsteins had the greatest proportion of MHC-I fibers (P < 0.01). Continental-influenced × dairy had the greatest proportion of MHC-IIA fibers (P < 0.01) and the greatest cross-sectional area of MHC-IIA, MHC-IIX (P < 0.01). Trained color panel attributes indicated strip loin steaks from carcasses of Angus sired beef × dairy crossbreds were the brightest, cherry-red throughout retail display (P < 0.01). Shear force values trained sensory analysis and consumer sensory analysis reported strip loin steaks from Holstein carcasses were the most tender (P < 0.01). No meaningful flavor differences were observed among sire breed type of beef × dairy crossbreds.

**Conclusion:** Thus, Continental sires can be used to improve carcass merit and red meat yield, whereas Angus sires can be used to generate more favorable retail acceptability by improving color sustainability and when mating to Holstein dams.

Keywords: beef, color, dairy, fiber type, sensory



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#### EFFECTS OF GRAIN AND GRASS-FED DIETS ON CARCASS CHARACTERISTICS AND MEAT QUALITY ATTRIBUTES OF BEEF STEERS

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**Objectives:** Recently, the demand for grass-fed beef boosted its market growth, which is expected to grow globally by USD 14.50 billion during 2020-2024. In addition, the demand for local beef has also been increasing as consumers raise concerns about where food has been sourced from. Thus, local farmers located on east and west coasts have been finishing beef with grass diets to explore niche markets. In this study, we evaluated the effects of grain and alfalfa-only diets on marbling score, yield grade and meat quality attributes of steers.

**Materials and Methods:** Twenty-four steers weighing approximately  $384 \pm 12.14$  kg were randomly assigned to 1 of 2 dietary treatments (grain-fed n = 12, and grass-fed n = 12 animals per treatment). Diets were formulated with 80% corn and 20% alfalfa hay (16% CP – Grain Fed); and 100% alfalfa hay (21% CP – Grass-fed). All animals were fed individually and offered *ad libitum* amounts of water. After 120 days, animals were transported and slaughter at a commercial beef processing plant. Approximately 24h postmortem, the yield grade, ribeye area, and marbling score were obtained directly from the plant's electronic instrument grade augmentation system. The short loin was removed from the carcasses and transported under refrigeration to Wolf Pack Meats, the University of Nevada USDA processing plant. After 7 days, strip loin steaks (1 in.) were fabricated and displayed for 7 days for color and lipid oxidation analysis. One steak was used for proximate analysis and two steaks were used to estimate lipid oxidation. One steak was vacuum packaged at day 7 and the steak used for color analysis (CIE L\*, a\*, and b\*) was vacuum packaged after the end of the display and used for lipid oxidation analysis at day 14. Steaks used for proximate analysis (AOAC, 2005) were vacuum packaged at day 7. Steaks used for Warner-Bratzler Shear Force (WBSF) were frozen at day 7 d (7d aging) and day 14 (14 d aging). Data was analyzed as a completely randomized design where color and lipid oxidation data were evaluated as a repeated measure. Data were analyzed using the CORR and GLIMMIX procedures of SAS. Means were considered different at  $P \le 0.05$ .

**Results:** Dietary treatments did not affect fat, ribeye area, yield grade, lipid oxidation, WBSF, and b\*. Moisture (*P*<0.0001) and marbling (*P*=0.0009) values were higher in steaks from steers feed grain than for those fed grass. A moderate positive correlation was observed for fat and marbling (r=0.64, *P*=0.0008). Samples from animals fed grain were lighter (P=0.0461) and redder (*P*<0.0001) than samples from animals fed grass. Samples aged 14 days had higher values for MDA/Kg than samples aged 7 days (*P*<0.0001). As expected, steaks aged 14 days were more tender than steaks aged 7 days (*P*<0.0001). For L\*, no effect of display was observed on samples. For a\*, samples were redder on days 3 and 4 when compared to samples on day 7 of display. For b\*, a slight increase was observed from samples display for 7 days when compared to day zero.

**Conclusion:** Animals fed grain had greater marbling deposition than animals fed alfalfa diets. Overall, beef finished with alfalfa hay for 120 days with levels of crude protein about 21% may provide similar yield grade, lipid oxidation, and slight color differences when compared to grain-fed beef.

Keywords: beef, grass-fed, meat quality



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#### NUTRIENT PROFILE OF USDA PRIME BEEF CUTS

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**Objectives:** USDA Prime beef has increased from 3.3% of graded beef carcasses in 2000 to 10.3% in 2020. Accurate nutrient information is needed for USDA Prime beef cuts as they are becoming more available in retail and foodservices settings. Additionally, USDA beef cuts are not well represented by current data in the USDA National Nutrient Database for Standard Reference. The objective of this study was to provide a full and accurate nutrient profile for frequently purchased USDA Prime beef cuts.

**Materials and Methods:** Strip loin steaks, tenderloin steaks, ribeye steaks, top sirloin steaks and rib roasts (IMPS # 1180, 1189, 1112, 1184 and 108; respectively) from USDA Prime beef carcasses were collected from 6 geographical locations during 3 seasonally spaced collections. Raw and cooked evaluations were done on retail cuts (N = 180; 5 cuts × 6 locations × 3 collections × raw or cooked). For cooked evaluation, steaks were pan grilled and rib roasts were oven roasted. All cuts were dissected into separable lean, internal fat, external fat and refuse components. For laboratory analyses, separable lean of each cut type, from each evaluation (raw or cooked) and from each location were homogenized and composited per collection (n = 3 per cut and cook type). Raw ribeye steaks and rib roasts were composited together, while cooked ribeye steaks and rib roasts were composited separately due to different cooking methods. Internal and external fat of all cut types from each evaluation (raw or cooked) and each collection were composited together. Proximate composition (crude protein, lipid, moisture and ash), fatty acid profile, amino acid profile, mineral content, cholesterol content, and fat soluble plus B-vitamin content was determined for each composite (N = 33).

**Results:** Compared to USDA Choice beef cuts in the USDA National Nutrient Database for Standard Reference, each of the 5 USDA Prime beef cuts evaluated in this study had a greater percentage of total lipid. As a result, water soluble vitamins and minerals were decreased in Prime beef. Nonetheless, all Prime cuts still qualified as an excellent source of total protein, zinc, niacin, and Vitamin B12 – on a raw, separable lean basis (containing greater than 20% of daily values as established in the Dietary Reference Intakes). Additionally, tenderloin steaks, ribeye steaks, and top sirloin steaks classified as a good source of iron (containing 10 to 19% of daily values as established in the Dietary Reference Intakes). Prime tenderloin and top sirloin steaks contained less than 10 g of total fat – on a raw and separable lean basis, qualifying them as lean beef cuts. Fatty acid analysis determined 18:1(Oleic Acid), 16:0 (Palmitic Acid), 18:0 (Stearic Acid) and 18: 2 n-6 (Linoleic Acid) in the greatest concentrations; respectively. Of the fatty acid fraction, "heart healthy" monounsaturated fatty acids comprised 51%, 47%, 49% and 48%; respectively, in the raw, separable lean portions of strip loin steaks, top sirloin steaks and rib roasts.

**Conclusion:** USDA Prime beef is a good and excellent source for protein, as well as various essential nutrients. Although the total lipid content is greater in Prime versus Choice beef, the fatty acid composition offers a good amount of beneficial monounsaturated fatty acids. Nutrient data from this study may be used to update the USDA Nutrient Database, which is pertinent for educating consumers, health professionals, dietitians, and legislators.

Keywords: Beef, prime, nutrition, protein, fatty acids



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VALIDATING THE ABILITY OF RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY (REIMS) TO DIFFERENTIATE LAMB FLAVOR PERFORMANCE BASED ON CONSUMER PREFERENCE

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**Objectives:** The objective of this study was to investigate the capability of rapid evaporative ionization mass spectrometry (REIMS) to accurately identify and predict cooked sheep meat flavor and carcass characteristics based on consumer response utilizing metabolomics data acquired from raw samples.

**Materials and Methods:** Boneless leg samples (n=200) were collected from sheep representing two age classifications (n = 99 lamb, n =101 yearling), at three USDA harvest facilities located in California and Colorado. Collections were completed between the months of September 2019 to January 2020. Consumer sensory panels (2019-590) consisted of 200 panelists who answered a series of questions regarding flavors and overall liking of ground sheep patties. REIMS platform captured metabolomics data from lean tissue, external fat tissue and ground patties. New binary values were developed, and levels of intensity based on consumer responses developed predictive models. Principal component analysis (PCA) reduced data dimensionality prior to creating the model using linear discriminant analysis (LDA). Additionally, Top 100 REIMS bins were determined by an f-statistic value before creating the model using LDA. Moreover, Top 100 REIMS bins were entered into UCSD Metabolomics Workbench to determine possible compounds responsible for sheep characteristics and flavor attributes (Metabolomics Workbench, University of California San Diego, San Diego, California, USA).

**Results:** Results show Top 100 REIMS bins were more effective overall when classifying attributes and predicting overall accuracy. Overall accuracies were above 80% for all sheep flavor attributes and characteristics with production background (grain-fed or grass-fed) revealing up to 100% classification accuracies. In comparison, PCA-LDA model revealed lower overall accuracies for all attributes. However, PCA-LDA model classified production background (grain-fed or grass-fed) at high overall accuracies with lean tissue at 97.50%, external fat tissue at 95.00% and ground sensory patty at 97.50%.

**Conclusion:** Promising results were discovered from the metabolomics database, possible compounds from previously published literature correlated to sheep flavor attributes and characteristics.

Keywords: Lamb Flavor, Lamb Quality, Metabolomics, REIMS



#### Meat and Poultry Safety

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#### WHOLE GENOME SEQUENCING STUDY OF SHORT-TERM EVOLUTION OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI O157:H7 IN CATTLE

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**Objectives:** Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is a foodborne pathogen that cattle are known to harbor. The objective of this study was to understand how the genomes from four different STEC O157:H7 strains inoculated into cattle at the rectal anal junction changed over 31 days.

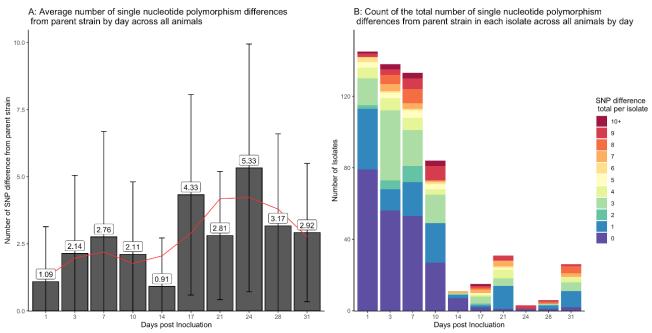
**Materials and Methods:** At the start of the study, sixteen cattle were randomly divided into four groups and sampled for STEC O157:H7 then subsequently inoculated at the rectal anal junction with one of four STEC O157:H7 parent strains: two *tir* 255 T>A T allele strains (NE1127 and NE1092) and two *tir* 255 T>A A allele strains (TX376 and NE1040). Strains with different *tir* alleles were chosen due to this polymorphism's associated with propensity to cause human clinical disease. After inoculation, STEC O157:H7 strains were recovered on ten different days over the course of 31 days through traditional culture enrichment and plating techniques. STEC O157:H7 was recovered from all animals with the highest numbers found on days 1 to 10, and through enrichment on days 14 to 31. Short-read sequencing was conducted on 592 strains from 14 animals that passed quality control.

**Results:** Recovered strains from all animals were compared to their parental inoculant strain. Most exhibited little to no change from their parental strain, with 38.7% containing no single nucleotide polymorphisms (SNPs) in the core genome and an additional 19.3% and 17.7% containing 1 or 2 SNPs, respectively, for a total of 75.7% of all strains having 2 or fewer SNPs. Among all strains recovered, there were 322 specific gene locations where SNPs were identified. Of these SNPs, 232 (72%) only occurred in one strain at one location. Finally, there were 24 SNPs that occurred within multiple animals inoculated with the same parental strain, representing 10 genes (two with multiple SNPs within the same gene), and four intergenic regions (one with four SNPs over an 11 base pair length).

**Figure: (A)** average number of single nucleotide polymorphism (SNP) differences of a recovered strain versus its inoculant parent across all animals **(B)** count of the total number of SNP differences from the inoculant parent stain by day across all recovered strains.

Image:





**Conclusion:** Observing variation among different strains of STEC O157:H7 in a bovine colonization model contributed to understanding short-term genetic changes of this pathogen in its natural host.

Keywords: E coli, Escherichia coli O157:H7, Shiga toxin producing Escherichia coli, WGS



#### Meat and Poultry Safety

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#### ANTIMICROBIAL EFFICACY OF CHEMICAL TREATMENTS AGAINST TWO INOCULATION LEVELS OF SALMONELLA ENTERICA ON PORK JOWLS

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**Objectives:** Salmonella is responsible for more than 1.3 million infections in the U.S. every year and is the leading cause of foodborne illness-related hospitalizations (26,500/year) and deaths (420/year). Livestock, such as swine, are usually asymptomatic carriers of Salmonella, which can lead to a high prevalence of this pathogen at the farm that can be carried to the abattoir. A 13.6% Salmonella prevalence has been reported on raw pork products collected from federally inspected slaughter and processing establishments in the U.S. Nevertheless, research on novel antimicrobial interventions for decontamination of pork carcasses and products is limited. Therefore, the objective of this study was to evaluate the decontamination efficacy of various chemical treatments when applied to pork jowls inoculated with a high or low contamination level of Salmonella enterica.

**Materials and Methods:** Chilled pork jowls were cut into  $10 \times 5 \times 1$  cm portions and were surface inoculated on the skin side with a mixture of six *S. enterica* serotype strains. Inoculation levels targeted were 6 to 7 log CFU/cm<sup>2</sup> (high) and 3 to 4 log CFU/cm<sup>2</sup> (low). Inoculated samples were left untreated (control) or were treated by spray application (10 s, 18 to 19 psi, 1.0 gpm flow rate) with water, a sulfuric acid and sodium sulfate blend (SSS, pH 1.2), formic acid (1.5%), peroxyacetic acid (PAA, 400 ppm), or PAA (400 ppm) that was pH-adjusted (acidified) with acetic acid (1.5%), formic acid (1.5%), or SSS (pH 1.2). Samples were analyzed for *Salmonella* populations immediately after treatment application (0 h) and after 24 h of refrigerated (4°C) storage. The study was designed as an 8 (treatments) × 2 (sampling times) factorials for each inoculation level (high, low), blocked by trial day. The experiment was repeated on two separate days for each inoculation level, and three samples were analyzed per treatment and sampling time in each trial (n = 6 samples per treatment and sampling time). Data were analyzed using the emmeans package in R (version 3.5.1). Least-squares means were separated using a significance level of  $\alpha = 0.05$ .

**Results:** Overall, initial pathogen levels on jowls inoculated at the high (6.2 log CFU/cm<sup>2</sup>) and low (3.5 log CFU/cm<sup>2</sup>) contamination level were reduced (P < 0.05) by 1.2 to 1.9 log CFU/cm<sup>2</sup> and 1.0 to 1.7 log CFU/cm<sup>2</sup>, respectively, following spray application of the tested chemical treatments. For the high inoculation level, *Salmonella* counts of samples analyzed after 24 h of refrigerated storage were, in general, similar ( $P \ge 0.05$ ) to the counts of the corresponding treatment at 0 h. However, for samples inoculated at the low inoculation level, pathogen counts recovered from jowls treated with SSS, formic acid, or PAA acidified with formic acid, and held at 4°C for 24 h, were 0.6 log CFU/cm<sup>2</sup> lower (P < 0.05) than the 0-h counts of the corresponding treatment. Regardless of inoculation level or sampling time, no ( $P \ge 0.05$ ) differences in efficacy were obtained between PAA and any of the acidified PAA treatments evaluated.

**Conclusion:** All evaluated chemical spray treatments effectively reduced both the high and low *Salmonella* contamination levels on pork jowls. Under the conditions of the study, acidification of PAA with 1.5% acetic acid, 1.5% formic acid, or SSS (pH 1.2) did not enhance the bactericidal effects of PAA.

Keywords: Antimicrobials, Peroxyacetic acid, Pork, Salmonella enterica



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### THE EFFECT OF A DIRECT-FED MICROBIAL (10-G) ON SALMONELLA PREVALENCE IN LYMPH NODES AND FECES OF FED BEEF HEIFERS

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**Objectives:** Salmonella is a naturally occurring bacteria that is known to cause upwards of 1.35 million cases of foodborne illnesses annually. Ground beef products may be manufactured from trimmings containing Salmonella infected lymph nodes, which has led to pending rulemaking by USDA-Food Safety and Inspection Service to declare Salmonella as an adulterant. Direct fed microbials are a pre-harvest intervention for reduction of Salmonella. The objective of this study was to determine the efficacy of a direct-fed microbial upon the prevalence and enumeration of Salmonella in feces and lymph nodes.

Materials and Methods: Heifers (n=1,394; 291 ± 9.9 kg) were blocked by day of arrival and randomly allocated to one of two treatments (0 or 2g/animal/d; CON and 10-G, respectively) with ten pens per treatment. Heifers fed 10-G were provided 1 billion CFUs per animal per day of Lactobacillus acidophilus, Enterococcus faecium, Pediococcus pentosaceus, Lactobacillus brevis and Lactobacillus plantarum. Twenty-four animals were randomly selected from each pen for Salmonella sampling. Rectoanal mucosal swab samples (RAMs) were obtained at initial processing and harvest; subiliac lymph nodes were collected at harvest. In addition, pen surface fecal pats were collected and composited by pen (10 pats per composite, 5 composites per pen) on days 0, 52, 120 and 170. Microbiology data was analyzed using the GLIMMIX procedure of SAS with pen as experimental unit and block as random effect. **Results:** Salmonella prevalence of RAMs did not differ between treatments at initial processing (P = 0.92; CON = 11.6%, 10-G = 11.5%) or at harvest (P = 0.92; CON = 99.0%, 10-G = 98.6%), however RAMs differed (P < 0.01) in Salmonella prevalence between the two collection times. Likewise, Salmonella log (mpn/g) of RAMs did not differ between treatments at initial processing (P = 0.63; CON = 0.28, 10-G = 0.30) or at harvest (P = 0.63; CON = 4.40, 10-G = 4.05), while log (mpn/g) of Salmonella increased (P < 0.01) over the feeding period. Moreover, composited pen level fecal pats were similar for Salmonella prevalence (P = 0.73; CON = 69.0%, 10-G = 67.0%) between treatments, but prevalence increased (P < 0.01) sharply during the initial 52 d then plateaued during the remainder of the finishing period. However, Salmonella prevalence differed (P < 0.01) among sampling days. Cattle fed 10-G had a lower frequency of Salmonella positive lymph nodes (P = 0.01; CON = 15.80%, 10-G = 7.41%) than CON. However, Salmonella

**Conclusion:** This data indicates that cattle fed 10-G had fewer *Salmonella* positive lymph nodes, which in turn can likely improve public health by reducing the number of foodborne illnesses caused by *Salmonella*.

log (mpn/g) of lymph nodes did not differ between treatments at harvest (P = 0.34; CON = 0.73, 10-G = 0.34).

Keywords: direct-fed microbial, heifers, Salmonella



### A NITRITE-ALTERNATIVE INGREDIENT INHIBITS CLOSTRIDIUM PERFRINGENS IN A MODEL MEAT SYSTEM DURING EXTENDED COOLING

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**Objectives:** USDA-FSIS released guidance documentation regarding compliance guidelines for cooling heat-treated meat and poultry products, known as Appendix B. Products, formulated with inhibitory ingredients may be qualified for extended cooling. Here, a novel cultured cane sugar and vinegar (CCSV) ingredient, designed as a nitrite-replacement product, is validated to inhibit *Clostridium perfringens* in a meat model system.

**Materials and Methods:** Three strains of *C. perfringens* spores were prepared and added to raw meat batter, comprised of ground pork *Semimenbranosus* and treatment. If necessary, sodium bicarbonate and carrageenan were added to product formulation to achieve target pH ( $6.3 \pm 0.05$ ), moisture ( $72 \pm 0.07\%$ ), and salt levels ( $2.0 \pm 0.25\%$ ). Treatments were comprised of a negative control (no treatment), positive control (100 ppm sodium nitrite: 250 ppm sodium erythorbate), and three levels of CCSV (1.9%, 2.2%, and 2.5%). Spores were added to meat samples at target level 2.5 log CFU/g and mixed for 3 min. Samples were portioned (50 g) into bags, vacuum packaged, pressed to uniform thickness (3 mm), and stored ( $4^{\circ}$ C) until heat treatment. Upon heating, samples were placed in water baths ( $75^{\circ}$ C) to heat shock the spores (target  $73^{\circ}$ C product temperature), then were removed from water bath and subjected to biphasic cooling. Two cooling profiles were analyzed: modified cooling phase (54.4 to  $26.7^{\circ}$ C in 2.5 h; 26.7 to  $7.2^{\circ}$ C in 10 h) and Appendix B Option 3 (54.4 to  $26.7^{\circ}$ C in 5 h; 26.7 to  $7.2^{\circ}$ C in 10 h). Samples were enumerated, in triplicate, at 0-time (immediate post-cook), 2.5, 5, 7.5, 10, and 15 h. Samples were diluted in 50 mL Bufferfield's buffer and stomached for 2 min. Samples were diluted and spread plated on tryptose-sulfite-cycloserine agar (TSC) with a TSC overlay (anaerobic incubation,  $35^{\circ}$ C, 24 h). Two trials were conducted.

**Results:** Application of CCSV inhibited outgrowth of *C. perfringens*. The negative control displayed growth rate and pattern similar to predictions made using ComBase. The positive control and all CCSV treatments showed no growth of *C. perfringens*, with a decrease in counts often recorded. Specifically, in Trial 1 modified cooling profile (total time 12.5 h), the uncured control exceeded 1.0 log CFU/g outgrowth by 7.5 h, while the cured control and treatments (1.9%, 2.2%, and 2.5% CCSV) resulted in population changes of -0.6, -1.11, -1.15, and -1.02 log CFU/g, respectively, at 12.5 h. For the Option 3 cooling profile, the uncured control exceeds 1.0 log CFU/g outgrowth at 2.5 h, 2.0 log CFU/g outgrowth at 5 h, and 4.0 log CFU/g outgrowth at 7.5 h. Cured control and treatments resulted in population changes of -1.06, -1.09, and -1.30 log CFU/g, respectively at 15 h. For Trial 2, results for the modified cooling profile showed greater than 1.0 log CFU/g outgrowth in negative control by 7.5 h, while the cured control and treatments resulted in changes of -0.90, -0.85, -1.01, and -0.97 log CFU/g, respectively at 12.5 h. Results for Trial 2 Option 3 Cooling is given in the table. In both trials, traditional curing and CCSV resulted in significant (P<0.05) population changes compared to negative control.



### Table 1. Average log change (CFU/g) of *Clostridium perfringens* in model meat system over 15 hour cooling period (Appendix B, Option 3)

	Treatment					
Timepoint	Uncured	Cured Control	1.9% CCSV	2.2% CCSV	2.5% CCSV	
(h)	Control					
0	-0.11 ± 0.08ª	-0.38 ± 0.02 <sup>b</sup>	-0.39 ± 0.06 <sup>b</sup>	-0.44 ± 0.13 <sup>b</sup>	-0.53 ± 0.14 <sup>b</sup>	
2.5	1.34 ± 0.21ª	-0.52 ± 0.06 <sup>b</sup>	-0.66 ± 0.04 <sup>b</sup>	-0.71 ± 0.10 <sup>b</sup>	-0.63 ± 0.03 <sup>b</sup>	
5.0	2.86 ± 0.12ª	-0.53 ± 0.07 <sup>b</sup>	-0.65 ± 0.06 <sup>bc</sup>	-0.62 ± 0.08 <sup>bc</sup>	-0.82 ± 0.05°	
7.5	3.95 ± 0.06ª	-0.65 ± 0.16 <sup>b</sup>	-0.56 ± 0.04 <sup>b</sup>	-0.70 ± 0.14 <sup>bc</sup>	-1.03 ± 0.21 <sup>c</sup>	
10.0	$4.18 \pm 0.06^{a}$	-0.59 ± 0.15 <sup>b</sup>	-0.92 ± 0.17 <sup>b</sup>	-0.78 ± 0.15 <sup>b</sup>	-0.82 ± 0.05 <sup>b</sup>	
15.0	4.37 ± 0.05ª	-0.72 ± 0.08 <sup>b</sup>	-1.42 ± 0.10 <sup>c</sup>	-0.83 ± 0.13 <sup>b</sup>	-0.80 ± 0.21 <sup>b</sup>	

Superscripts within rows with different letters are significantly different (P<0.05).

**Conclusion:** Application of CCSV inhibits the outgrowth of *C. perfringens* in a model meat system, achieving food safety standards outlined in Appendix B. Use of CCSV can serve as an alternative food safety system in replacement of sodium nitrite.

Keywords: Appendix B, Clostridium perfringens, cooling



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### EFFICACY OF LACTIC ACID INTERVENTIONS AT AND ABOVE REGULATORY ALLOWABLE UPTAKE LEVELS ON THE REDUCTION OF PATHOGENIC BACTERIA

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**Objectives:** In fresh ground products, pathogenic bacteria such as *Salmonella* and shiga toxin producing *E. coli* can be reduced by organic acid interventions. Currently, fresh beef trim processors are allowed a maximum uptake percentage of 0.49% added weight post intervention application. The objective of this study was to evaluate the efficacy of lactic acid (4.5%) intervention uptake  $\leq$  to 0.49% and above. Uptake percentages were achieved through both spray and dip applications of lactic acid on fresh beef trim.

**Materials and Methods:** For this study, seven strains of Shiga toxin producing *Escherichia coli* (STEC) (O26, O103, O45, O111, O121, O145, and O157:H7) and three strains of *Salmonella* (*Salmonella* Typhimurium, *Salmonella* Newport, and *Salmonella* Enteritidis) were used to inoculate beef trim samples. Beef trim of two different lean levels (90/10 and 50/50) were collected from a commercial meat processing facility. Beef trim size was reduced by cutting to achieve 20-gram pieces (± 2 grams) and 46 pieces were placed in a 22x28cm bag, then distributed on a tray covered in foil. Trim pieces were then inoculated with the *E. coli* or *Salmonella* strains using a multi-purpose spray bottle (15psi) and left for 20 minutes to allow bacteria to attach. Trim pieces were weighed before and after the application of the intervention to calculate uptake percentage. The spray and dip applications were applied with a 4.5% lactic acid solution to trim pieces in 1, 5, and 10 second intervals. After the interventions were applied, trim pieces stayed on the tray for 1 minute, then were placed into a bag for 5 minutes to simulate a combo bin at a commercial processing facility. After the trim was weighed post- intervention, the pieces were transferred to a 2L filtered whirl-pack bag and 500 mL of Buffered Peptone Water (BPW) was added, then mixed for 30 seconds to have a homogenous sample. Meat rinsates were serially diluted, plated in triplicate, and incubated for 18-24 hours at 37°C. Bacterial counts were log transformed for statistical analysis. A regression analysis was performed to observe the relationship between uptake percentage and the reduction of pathogenic bacteria.

**Results:** In the regression model used for this study, for every 1% increase in uptake percentage, there was an average reduction of 0.16 LogCFU/g for STEC and *Salmonella* using lactic acid interventions (P < 0.01). In the 50/50 trim, there was an increase in the reduction of *Salmonella* by 0.11 LogCFU/g (P=0.05) and a decrease in the reduction of STEC by 0.03 LogCFU/g ( $P \ge 0.46$ ) for every 1% increase in uptake percentage. In the 90/10 trim, there was a decrease in reduction in *Salmonella* by 0.10 LogCFU/g (P=0.15) and STEC by 0.26 LogCFU/g ( $P \le 0.004$ ). **Conclusion:** This study found that an increase in uptake percentage using a organic acid intervention significantly

reduced pathogenic *Salmonella* and *E. coli* bacteria in fresh beef trim. Further research should be done at commercial processing facilities to see if the same results can be found when done on a larger scale.

Keywords: antimicrobial, bacteria, pathogen, uptake level



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### EVALUATION OF THE AVIAN, BOVINE, AND PORCINE FECAL MICROBIOME AND THEIR EFFECT ON SUBSEQUENT MICROBIAL ENVIRONMENTS

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**Objectives:** Animal feces are biological contaminants of concern for the meat industry due to the potential to adulterate meat products during harvest. Fecal waste is also commonly applied as amendments to crops, providing another potential route to enter the food supply chain. While fecal contamination of meat products has the potential to be a public health hazard, many consumers view the use of animal waste as a fertilizer to be more beneficial than the application of chemicals to crop land. The extent that fecal microbial communities can impact the microbiomes of meat products and soil needs further investigation. Therefore, the objective of this study was to use 16S rRNA gene sequencing to characterize how fecal waste from different meat animal operations was associated with the microbiome of meat rinsates and the microbiome of soil where fecal waste was used as an amendment.

**Materials and Methods:** Composited fecal samples were collected from three commercial operations: beef feedlot, finishing swine operation, and broiler chicken operation (n=20/species). Meat rinsates (n=20/species) were obtained from the harvest of the animals from the same facilities. After feces or litter (from the same cohorts of animals) was composted and spread, soil samples (n=20/species) were collected from fields. Additionally, human waste samples were collected from wastewater treatment plants in close proximity to each production operation (n=14/species). DNA was extracted from samples using the PowerMax Soil DNA Isolation Kit according to the manufacturer's protocol. The V4 region of the 16S rRNA subunit was PCR amplified with the 515F/806R primer pair, and sequencing (2 x 250bp) was conducted on a HiSeq 2500. Microbiome data was analyzed using QIIME2. Reads were denoised using DADA2, and taxonomy was assigned using a pretrained Naïve Bayes classifier Diversity metrics and differences were calculated using R and QIIME2. SourceTracker2 was used to determine to the extent that the fecal microbiome influenced the associated environmental microbial communities.

**Results:** Ten million reads were classified taxonomically to 6,868 amplicon sequence variants (ASVs) with an average of 52,841 reads per sample. Shannon's Diversity Index was different (*P*<0.05) within species for both avian and bovine sampling locations, respectively. Additionally, within porcine samples, soil and wastewater samples had similar (*P*>0.05) Shannon's Diversity Indexes that were greater (*P*<0.05) than fecal and meat samples. Weighted and unweighted UniFrac values clustered by environment (fecal, meat, soil, or wastewater), indicating the microbial communities associated with each environment were different (*P*<0.05). This was further supported by the SourceTracker2 report, which showed that a low percentage of the environmental samples (meat, soil, wastewater) could be sourced back to feces, regardless of species.

**Conclusion:** The differences in microbial community structure between fecal samples and their subsequent environments in all species, and the low percentage of environmental communities attributed to fecal origins suggest that animal waste has minimal influence on the microbial communities of associated environments. Ultimately, this could provide additional evidence that there is a minimal concern of pathogen introduction or increased antimicrobial resistance from fecal applications.

Keywords: Beef, Chicken, Fecal Microbiome, Human, Pork



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### EFFECTIVENESS OF ORGANIC ACIDS ON THE FATE OF ESCHERICHIA COLI ATCC BAA-1427 IN FRESH GROUND PORK PATTIES

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Objectives: Fresh pork products are susceptible to pathogen contamination during processing. Organic acids have been evaluated as effective antimicrobials that can be used in the meat industry. The objective of this study was to determine the effectiveness of malic and tartaric acid as antimicrobials in ground pork patties compared to acetic acid. Materials and Methods: Surrogate nonpathogenic E. coli ATCC BAA-1427 was hydrated by adding 1mL of trypticase soy broth (TSB) into a vial containing a lyophilized culture and transferring it into a 15mL conical falcon tube, and incubated for 24h at 37°C. An aliquot of the surrogate microorganism was taken from the incubated culture and plated on an agar plate by quadrant streaking and incubated for 24h at 37°C to ensure culture purity. A single, isolated colony was used to inoculate 5 mL of TSB to generate another overnight culture for use in the study. An initial batch of ground pork (7,000 g) was inoculated with 3500uL of E. coli ATCC BAA-1427. Pork rested for 1h at 4°C to allow for attachment and then mixed by hand for 5 min to allow for dispersion. Inoculated pork was separated into seven 907 g batches, and treatments were applied. There were seven treatments: a negative control, a positive control (acetic acid), malic, and tartaric acids at either 1.5% or 3% concentration rate. Each batch was then re-mixed for 3 min to allow dispersion of organic acids. Two replications of 6 patties (149 g) per treatment were placed on Styrofoam trays and overwrapped with polyvinyl chloride film, arranged into a retail display case maintained at 2°C for 12 days. Pork patties were subjected to microbial collection on d 1, 2, 4, 6, 8, 10, and 12. Five g microbial samples were placed into a sterile stomacher bag filled with 195mL of DI water, and homogenized for 30 sec. Serial 5-fold dilutions were done and plated on eosin methylene blue (EMB) agar plates, spread evenly across the surface using a sterile inoculating loop, and incubated for 24h at 37°C. Colonies were enumerated and reported as colony forming units (CFUs). Statistical analysis was performed using the F-Protected t-Test in SAS v. 9.4 (SAS Institute, Cary, NC). The analysis of variance was analyzed using time as the repeated measure, sample number as the subject, treatment as the fixed effect, and rep as the random effect. Separation of means was performed by using the least significant differences (LSD) in Saxton's PDMIX800 macro. Differences were reported at  $\alpha$ =0.05.

**Results:** There were no differences (P > 0.05) noted among treatments on the presence of *E. coli* in patties. However, differences were found in *E. coli* populations over retail display. On d 6, *E. coli* populations were significantly the highest (354 CFUs/1ml/5g). Additionally, *E. coli* populations declined (P < 0.05), following d 6 to similar values (P > 0.05) as d 1 and 2 of retail display.

**Conclusion:** Malic and tartaric acids resulted in similar *E. coli* populations compared to acetic acid over retail display. There is potential for tartaric and malic acids to be used as effective antimicrobials, which could inhibit bacterial resistance.

Keywords: ATCC BAA-127, Escherichia coli, Ground pork, Organic acids



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### PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF E. COLI IN MARKET SWINE AND CATTLE COLLECTED FROM STOCK SHOWS PRE AND POST IMPLEMENTATION OF THE VETERINARY FEED DIRECTIVE

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**Objectives:** The objective of this study was to estimate the prevalence and characterize the antimicrobial susceptibility profiles of *E. coli* isolates obtained from 2020 show animals, Post-Veterinary Feed Directive (VFD) implementation and compare them to similar isolates collected in 2015, Pre-VFD implementation.

**Materials and Methods:** Fecal samples collected from swine (n = 40) and cattle (n = 40), were enumerated for *E. coli* using 3M *E. coli*/Coliform Count Plates, and four isolates/sample were isolated onto MacConkey Agar for further testing. Antimicrobial testing was conducted with a microbroth dilution method using Trek Diagnostic Sensititre plates. Isolates were tested against 14 antimicrobial agents important to human and animal health, including: Cefoxitin, Azithromycin, Chloramphenicol, Tetracycline, Ceftriaxone, Amoxicillin/Clavulanic Acid, Ciprofloxacin, Gentamicin, Nalidixic Acid, Ceftiofur, Sulfisoxazole, Trimethoprim/Sulfamethoxazole, Ampicillin, and Streptomycin. Resistance breakpoints used were established by Clinical Laboratory Standards Institute. Data was analyzed using procedures of SAS (9.3). Overall percent isolates resistant to antimicrobials was determined, as well as differences in Minimum Inhibitory Concentrations (MICs) between Pre- and Post-VFD samples.

**Results:** Of the 309 total Post-VFD isolates, 71.84% were resistant to at least one antimicrobial, while 80.30% of Pre-VFD isolates (n=662) were resistant to at least one antimicrobial. Of the 159 Post-VFD swine isolates, 80.50% were resistant to Tetracycline and 43.40% to Streptomycin; comparatively, of the 330 Pre-VFD swine isolates, 96.67% exhibited resistance to Tetracycline followed by 69.70% to Sulfisoxazole. Of the 159 Post-VFD cattle isolates, 48.00% exhibited resistance to Tetracycline and 26.67% to Streptomycin; comparatively, of the 332 Pre-VFD cattle isolates, 55.12% exhibited resistance to Tetracycline and 32.53% to Streptomycin.

A significant interactive effect between the main effects of sampling year and animal species impacted differences in MIC levels in seven of the 14 antimicrobials tested ( $P \le 0.05$ ). When evaluating differences between Pre- and Post-VFD swine isolates, a higher MIC level was observed in Azithromycin, Tetracycline, Sulfisoxazole, Ampicillin, and Streptomycin from Pre-VFD samples ( $P \le 0.05$ ); Cefoxitin, Ceftriaxone, Ciprofloxacin, and Ceftiofur had a higher MIC level in Post-VFD isolates ( $P \le 0.05$ ), and no difference was evaluated between Pre- and Post- isolates among the remaining five antimicrobials (P > 0.05). When evaluating Pre- and Post-VFD cattle isolates, Pre-VFD isolates exhibited a higher MIC level in Cefoxitin, Azithromycin, Tetracycline, Ceftiofur, and Streptomycin ( $P \le 0.05$ ), while no difference was noted in the remaining nine antimicrobials (P > 0.05). Overall, this study saw varying degrees of susceptibility across the antimicrobials tested.

**Conclusion:** No consistent pattern of change was seen in MIC levels from Pre- to Post- samples for the various antimicrobials studied. Given current regulations, the animal production industry should continue to investigate alternative options to previously used in-feed antimicrobials including livestock exclusive antimicrobials, alternative drugs, or non-antimicrobial methods of increasing feed efficiency for livestock.

Keywords: antimicrobial susceptibility, Escherichia coli, show cattle, show swine, Veterinary Feed Directive



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### LACTIC ACID AND SPOILAGE BACTERIA BIOFILMS INFLUENCE STEC PERSISTENCE ON FOOD CONTACT SURFACES AND TRANSFER TO BEEF

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**Objectives:** The impact of biofilm formation on STEC persistence is often investigated using single-species wet biofilms. However, this may not be representative of beef processing facilities, where spoilage (SP) or lactic acid bacteria (LAB) may form biofilms. This research aimed to evaluate potential synergistic and antagonism interactions of STEC within either LAB or SP wet and dry multispecies biofilms. It was hypothesized that pre-colonization of LAB and/or SP bacteria could reduce STEC adhesion and colonization of food contact surfaces and impede STEC biofilm formation. **Materials and Methods:** LAB and SP strains tested were selected based on their biofilm-forming ability. The STEC O103 serogroup was included in this research due to its high prevalence in western Canadian slaughter cattle. One LAB combination, **T1**: *Carnobacterium piscicola + Lactobacillus bulgaricus,* and two SP combinations **T2**: *Raoultella* sp. + *Comamonas* sp. and **T3**: *Pseudomonas aeruginosa + Comamonas sp.* were selected to form multispecies biofilm at 10 °C and 25 °C for 6 days on thermoplastic polyurethane (TPU) and stainless steel (SS) coupons. On day 6, STEC O103:H2 was added to the preformed biofilms and incubated for another 6 d. The O103 single-species biofilm was developed as control positive (**T4**). Coupons covered by mature biofilms (n=288) were washed with Butterfield Phosphate Buffer (BPB) and placed in a sterile Petri Dish, then randomly assigned to different factors as described in table 1. A group of SS and TPU coupons were designated to test STEC transfer to beef. The second set of coupons was used for STEC enumeration.

Factor level		
T1, T2, T3, T4		
10.25		
10, 25		
6, 30, 60		
, 50, 00		
Moist (60~90%), Dry (20% ~ 30%)		
TPU, SS-304		

Table 1. The factor and factor level detail for biofilm formation and meat transfer.

**Results:** At 25 °C, T3 biofilm was the most antagonistic against O103, achieving a 6  $\log_{10}$  cfu/g reduction (P < 0.0001). In addition, O103 remained associated with either single-species (T4) or multispecies biofilms (T1-2) after 60 days storage in moist conditions, causing up to 1.67 Log<sub>10</sub> cfu/g meat contamination. Among the different strain combinations, the transfer of O103 to beef samples was reduced (P < 0.05) with aged biofilms, regardless of the moisture or surface type. Approximately 50% of the dry T2 biofilms harboured O103 after 60 d.

At 10 °C, none (P > 0.05) of the biofilms were antagonistic to O103. With moist biofilms, O103 cell numbers on beef decreased (P < 0.001) from 2.5 at 6 d to 0.6  $\log_{10}$  cfu/g after 60 days. Regardless of the surface type, the transfer of O103 to beef from moist biofilms was at least 1.0  $\log_{10}$  cfu/g, higher (P < 0.0001) than from dry biofilms. With moist biofilms, the O103 transfer to beef from the TPU surface was higher (P < 0.001) than from biofilms on SS-304. After 60 days of



storage under dry conditions, biofilm T2 on TPU and SS-304 surface, enhanced O103 survival rate from the range of 0% to 33.33% to the range of 33.33% to 100% comparing with T4, respectively.

**Conclusion:** In conclusion, SP and LAB bacteria commonly found in the beef industry could play a significant role in STEC persistence, survival and cross-contamination. Thus, LAB and SP elimination and biofilm formation prevention on food processing environments is essential to prevent/reduce STEC persistence and survival.

Keywords: Multispecies Biofilm, Persistence, STEC



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FATE OF SALMONELLA IN BEEF STEAKS DURING SOUS VIDE COOKING
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**Objectives:** Sous vide is a method of preparing vacuum packaged food in hot water baths to achieve a precise degree of doneness throughout the food. It is commonly used in residential and food service kitchens; however, the microbiological safety of this cooking method has not been fully assessed. A previous experiment conducted in our laboratory in response to common cooking guidelines below USDA-FSIS Appendix A recommended time and temperatures demonstrated that sous vide cooking of steaks inoculated with generic E. coli did not achieve necessary bacterial reductions for safety at 46 °C. To further describe the potential safety risks associated with this cooking application, this experiment was conducted with a cocktail of Salmonella serovars. The objective was to evaluate the safety of beef steaks sous vide cooked to 54, 51, and 46 °C using Salmonella as the challenge organism. Materials and Methods: One-inch slices of beef semitendinosus muscles were vacuum packaged and frozen until use. For each replication, steaks were thawed (48 hours, 4º C) and exposed to UV light for 15 minutes on each side. Steaks were submerged in liquid inoculum (1 liter each of Salmonella Typhimurium, Enteritidis, and Heidelberg overnight culture) and inoculated to at least 7.4  $log_{10}$  with a 96 well inoculation pin pad inserted three times into each steak. Following inoculation, steaks were air-dried (30 minutes, 23°C), individually vacuum sealed, and cooked in sous vide water baths. Duplicate steak samples were taken from raw, inoculated steaks and at the following time and holding temperatures: 150 min/46 ºC, 420 min/46 ºC, 150 min/51 ºC, 193.5 min/51 ºC, 258 min/51 ºC, 322.5 min/51 ºC, 64.5 min/54 °C, 86 min/54 °C, and 107.5 min/54 °C. The 54 °C sampling at 86 minutes was taken directly from the USDA Appendix A 5 log<sub>10</sub> reduction table. The 51 and 46 <sup>o</sup>C sampling times were extrapolated from Appendix A and common recommended cooking times, respectively. Core samples (25 g) were homogenized with buffered peptone water, serially diluted, and plated onto xylose lysine deoxycholate agar. The Salmonella colonies were counted after incubation (24 hours, 35 °C) and reported as log<sub>10</sub> cfu/g. Reductions were determined by subtracting concentrations at given sampling times from the raw sample concentrations. Data were analyzed using PROC GLM contrasts in SAS 9.4. **Results:** The minimum time measured for a 5  $\log_{10}$  reduction for 54 °C cooking was 64.5 minutes (P < 0.01). The minimum time measured for a 5 log<sub>10</sub> reduction for 51° C cooking was 150 minutes (P < 0.01). Cooking at 51° C was also able to achieve a final reduction of 7.28  $\log_{10}$  (P < 0.01) after 322.5 minutes of holding. Cooking at 46° C achieved only a 2.01  $\log_{10}$  reduction (*P* < 0.01) after 420 minutes of holding.

**Conclusion:** Reductions of pathogenic *Salmonella* in this experiment demonstrate the validity of Appendix A time and temperature cooking combinations for meat products. Cooking non-intact products at 46° C is potentially hazardous. More data are still needed with other relevant foodborne pathogens to determine if sous vide cooking below Appendix A temperatures could lead to pathogen outgrowth.

Keywords: beef steak, Low Temperature, Salmonella, sous vide



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### EFFICACY OF PERACETIC ACID INTERVENTION AT AND ABOVE REGULATORY ALLOWABLE PICK-UP LEVELS FOR THE REDUCTION OF E. COLI AND SALMONELLA ON BEEF TRIM

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**Objectives:** Foodborne pathogens like *E. coli* and *Salmonella* are the leading cause of illness and even deaths worldwide. Numerous antimicrobial interventions are being used in processing plants to reduce pathogens. To consider these agents as processing aids, the retained water cannot exceed 0.49% addition to product weight. The objective of this study was to evaluate the food safety efficacy of peracetic acid at and above required uptake levels for processing aids through spray and dip applications.

**Materials and Methods:** A general linear regression was performed using the *Im* built in function of R for the analysis and *ggplot2* package for the data visualization (version 4.0.4). Where Log Reduction was used as the dependent variable and Uptake Percentage as the independent variable. A second linear regression model was performed having Log Reduction as the dependent variable. For the independent variables Uptake, Method of Application, and Trim were used, as well as the interaction of Uptake and the other explanatory variables. Beef trim was cut into 20g pieces and placed separately into a 22 × 28cm bag adding up to 46 pieces per bag. Afterwards, trim was placed evenly on a tray covered with aluminum foil and inoculated using a spray bottle with seven specific isolates of Shiga toxin producing *Escherichia coli* (STEC) (026, 0103, 045, 0111, 0121, 0145, and 0157:H7) or three *Salmonella* strains (Typhimurium, Newport, and Enteritidis), its weight was recorded prior to intervention (peracetic acid; 400ppm) through spray using a multi-purpose sprayer (15 psi with an acceptable spray pattern) or dip application for 1, 5, and 10 second intervals to obtain a broad uptake level from 0.10% up to 4.2%. Afterwards, intervened trim was placed into a 22 × 28cm bag to weigh and measure pick up level and then transferred to a 2L filtered whirl-pack bag where 500 mL of Buffered Peptone Water (BPW) was added. The trim was mixed to have a homogenous sample. Meat rinsates were serially diluted and plated following the drop dilution method and an enumerable range of 2-30 colonies was used to report results after log transformation.

**Results:** The regression model provided in this study showed that an increase of 1% in uptake percentage will increase the overall reduction by 0.16 LogCFU/g. There is a statistical significance in the reduction of *Escherichia coli* and *Salmonella* in relation to the uptake percentage (P < 0.01). When spray application is used, there is a decrease in the reduction of *Escherichia coli* by 0.68 LogCFU/g compared to dip application (P < 0.01). An increase of 1% in uptake will increase the reduction of *Salmonella* by 0.11 LogCFU/g. Both lean levels are statistically significant (P < 0.05), where 90/10 trim had a lower reduction than 50/50.

**Conclusion:** This study showed that interventions using organic acids such as peracetic acid at higher uptake levels, significantly reduces pathogens like *E. coli* and *Salmonella* in beef trim.

Keywords: antimicrobial, pathogen, processing aids, uptake level



### 110 APPLICATION OF VINEGAR BASED ANTIMICROBIALS INHIBIT THE GROWTH OF LISTERIA MONOCYTOGENES IN UNCURED TURKEY BREAST

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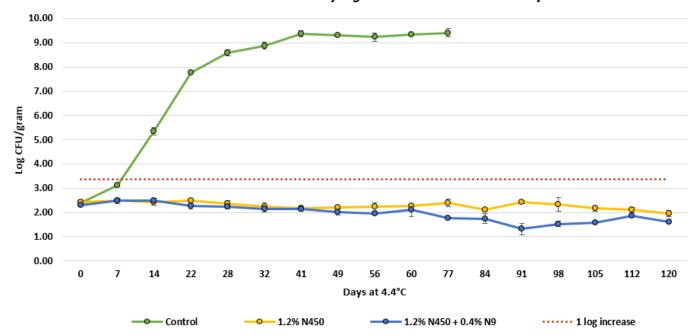
**Objectives:** Utilize application of vinegar-based antimicrobials in an uncured, sliced, turkey breast product to inhibit the growth of *Listeria monocytogenes* while maintaining sensory attributes.

**Materials and Methods:** Uncured, sliced, turkey breast was produced without antimicrobial, with 1.2% Verdad® Opti Powder N450 (Vin/SS), and with 1.2% Verdad® Opti Powder N450 + 0.4% Verdad® N9 (Vin). Turkey was sliced to a weight of 25 g, left uninoculated (for natural flora analysis) or inoculated with a *L. monocytogenes* cocktail, vacuum sealed, and stored at 4.4°C. At sampling, inoculated product was enumerated for *L. monocytogenes*, and uninoculated product was evaluated for natural flora interference and proximate analyses. To enumerate, turkey was transferred to a sterile stomacher bag, diluted 1:2 with buffered peptone water (BPW), and stomached at 200 rpm for 30 s. Serial dilutions were performed as necessary, and samples were spread plated, in duplicate on Modified Oxford Agar (MOX; 35°C incubation for 48 h) for *Listeria* and de Man, Rogosa, and Sharpe agar (MRS; 30°C incubation for 48 h) for background flora analysis. After 21 days of storage at 3.3°C, turkey samples were evaluated by a trained panel (n=10) for saltiness, bitterness, sourness, flavor intensity, tenderness, juiciness, and acceptability using a 7-point scale (1=no presence; 7=extreme presence; 9 panelists).

**Results:** Starting *L. monocytogenes* counts were ca. 2.4 log CFU/g. Negative control samples exceeded 2 log CFU/g outgrowth between sampling dates 7 and 14, while both treatments inhibited outgrowth throughout the 120-day study, with final populations of 1.95 and 1.62 log CFU/g in Vin/SS and Vin, respectively. Counts in both treatments were significantly (P<0.05) lower than negative control by day 7. There was no statistical (P $\ge$ 0.05) difference among treatments and control regarding sensory attributes. Statistics were performed using the ANOVA procedure (with Tukey procedure for population differences) in Minitab 18. **Image:** 



Listeria monocytogenes Growth in Deli Turkey



**Conclusion:** Application of Verdad<sup>®</sup> antimicrobials (1.2% Verdad<sup>®</sup> Opti Powder N450 and 1.2% Verdad<sup>®</sup> Opti Powder N450 + 0.4% Verdad<sup>®</sup> N9) inhibits the growth of *L. monocytogenes* in uncured turkey for 120 days while maintaining acceptable sensory characteristics.

Keywords: Food Safety, Listeria, shelf life, Turkey



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### EFFICACY OF REDUCING SALMONELLA PREVALENCE IN LYMPH NODES FROM INTRADERMALLY INFECTED GOATS BY CARCASS VASCULAR RINSING

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**Objectives:** To determine the ability of carcass vascularly rinsing (Rinse & Chill<sup>®</sup>, RC; MPSC Inc., Hudson, Wisconsin) to reduce *Salmonella* prevalence in lymph nodes from intradermally infected goats.

Materials and Methods: The experiment was conducted (IACUC A006428) over two different trial periods with cull dairy goats (N=20). The goats consisted of various breeds (Alpine, LaMancha, Nubian, Saanen, Cross), age (1.5 - 5 years), and live weight (71.2±14.1 kg). Goats with similar characteristics were grouped together in sets of 2 before being randomly assigned to 2 treatments (CN, not vascularly rinsed; n=10) or vascularly rinsed with a standard rinse solution (TRT: RC; 98.5% water; balance: glucose, polyphosphates, maltose; 9 °C; n=10). Prior to slaughter and treatment application, a lancet (ComforTen<sup>®</sup> Multiple Skin Test Device) was utilized intact (10LT; 10 surgical steel 1.2 mm lancet tips) or reduced to 3-lancet tips (3LT) to intradermally administer Salmonella serotype Enteritidis (SE13). The lancet was dipped into inoculum broth (6.8x10<sup>8</sup> CFU/mL) and then applied with light pressure (each leg with the 3LT, 13 applications; both anatomical sides of the caudal thorax near 12/13<sup>th</sup> thoracic vertebrae and ventral abdomen with the 10LT, 1 application). After a 7-day incubation period, the animals were stunned by penetrating captive bolt and one TRT was applied to each carcass immediately upon exsanguination. Carcasses were skinned, eviscerated, and the exterior was sprayed with an antimicrobial (5% lactic acid solution) before being hung in a cooler overnight. After chilling and prior to excising the lymph nodes (superficial cervical; medial iliac; subiliac; mammary), a chlorine bleach solution (400 ppm on chlorine) was applied to the carcass (3 min) followed by a cold-water rinse. Following aseptic procedures, the lymph nodes were dissected and trimmed to remove the majority of the non-lymphatic tissue prior to Salmonella enumeration and percentage moisture analysis. Animal served as the experimental unit. A 2 x 4 factorial design (TRT x lymph node) was used to statistically analyze the data and trial period served as a covariate in the analysis.

**Results:** TRT main effect on *Salmonella* counts was not significant (P=0.19; CN=3.82, RC=3.48, log CFU/g). A TRT x lymph node effect for counts was found (P<0.05; Least Significant Difference, 1.01 CFU/g). *Salmonella* counts in the medial iliac of RC (1.56 log CFU/g) were lower than in the CN (2.94 log CFU/g, P<0.05). No differences in counts (P>0.05) were found among the subiliac (4.39 log CFU/g), superficial cervical (4.29 log CFU/g) and mammary (3.68 log CFU/g). Percentage moisture in the medial iliac of RC (74.4%) was greater (P<0.05) than in the CN (68.7%). No differences (P>0.05) were found in the percentage moisture between CN and RC in the other lymph nodes. Perhaps the greater moisture content in the RC medial iliac suggests that more of the rinse solution reached this lymph node and therefore exposed it to more of the phosphates which are known to have antimicrobial activity.

**Conclusion:** The *Salmonella* infectivity model was successful by providing sufficient counts in the lymph nodes so that it could be used to assess the inclusion of other antimicrobial ingredients incorporated into the Rinse & Chill<sup>®</sup> solution. Rinse & Chill<sup>®</sup> was able to demonstrate a 1.3 log reduction in *Salmonella* but only in the medial iliac lymph node.

Keywords: Carcass Vascular Rinsing, Goat, Lymph Node, Salmonella Enteritidis



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### EVALUATION OF THE TEMPO <sup>®</sup> SEMI-AUTOMATED SYSTEM FOR THE ENUMERATION OF MICROBIAL INDICATORS: AEROBIC PLATE COUNTS (APC), ENTEROBACTERIACEAE (EB), COLIFORMS (CC), E. COLI (EC) AND LACTIC ACID BACTERIA (LAB) FOR MEAT AND POULTRY BIO-MAPPING STUDIES

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Objectives: To evaluate the performance of the bioMérieux Tempo® Semi-Automated System for the quantification of Aerobic Plate Counts (AC), Enterobacteriaceae (EB), Coliforms (CC), Escherichia coli (EC) and Lactic Acid bacteria (LAB) as compared to reference methods when used in bio-mapping studies in meat and poultry processing settings. Materials and Methods: Several microorganisms were selected to test five microbial indicators with high importance in spoilage and hygiene of meat and poultry products using the Tempo® Semi-Automated system Listeria innocua Seeliger ATCC 33091 for AC; Salmonella enterica subsp. enterica ATCC 14028 for EB; Citrobacter rodentium ATCC 51459 for Coliforms CC; Escherichia coli ATCC 25922 for EC, and Lactobacillus salivarius L28 for LAB. Each of the microorganisms were aseptically transferred into a tube with 9 mL of Tryptic Soy Broth (TSB) and incubated at 37 °C for 24h, except for the L28 that was incubated for 48h. Microorganisms were streaked on Tryptic Soy Agar (TSA) plates with no selective agents and placed into an incubator for at 37 °C for 24h, except for the L28 that was incubated for 48h under anaerobic conditions. Typical colonies were transferred with a cotton applicator into three tubes with 5 mL of sterile water and the concentration (1 ~ 2 × 10<sup>8</sup> CFU/ml) was verified using a nephelometer (0.5 McFarland turbidity). After reaching concentration, a cocktail combining the four standard microorganisms selected for AC, EB, CC and EC were obtained by placing them together into a 50 mL Falcon conical tube, and for LAB pure culture was used. Each culture was serially diluted using 9 mL Buffered Peptone Water tubes. Each dilution was treated as an independent sample for testing counts using the 3M<sup>®</sup> Petrifilms and the Tempo<sup>®</sup> System for AC, EB, CC, and EC, and MRS plates for LAB. For LAB, aerobic and anaerobic conditions were evaluated. Counts were log<sub>10</sub> transformed and statistically analyzed using R (Version 4.04).

**Results:** The slope of the linear models representing the rate of change in bacterial counts in one method due to an increment of 1 unit in the other method indicate minimal discrepancies in between methodologies. For the APC, EB, CC, and EC, the estimated slopes were 0.96, 0.98, 1.02, and 0.98 respectively. For the LAB evaluation, the estimated slopes were 1.063 and 0.970 for aerobic and anaerobic atmosphere incubation, respectively. All these values are not statistically different from 1, showing significant correlation in between methodologies.

**Conclusion:** The observed discrepancies in between methods are not significant in microbiological terms to indicate deviations from indicator levels in the samples evaluated. In general, for APC, the Tempo® system counts 0.5 Log<sub>10</sub> CFU/mL of sample higher than the reference methodology, but this can be explained by the different nature of the methodology, media needs and experimental execution, without affecting the comparability of the bio-mapping data. The bioMérieux Tempo® Semi-Automated System can be used for the quantification of microbial indicators in bio-mapping studies conducted with meat and poultry samples collected at different stages during processing.

**Keywords**: Aerobic Plate Counts, Coliform Counts, Lactic Acid Bacteria Counts, Microbial Enumeration Methods, Tempo System



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BIO-MAPPING OF PATHOGEN AND INDICATOR MICROORGANISMS AT DIFFERENT STAGES DURING CHICKEN PROCESSING WITH AND WITHOUT CHEMICAL INTERVENTIONS TO IDENTIFY STRATEGIC INTERVENTION POINTS AND DEVELOP STATISTICAL PROCESS CONTROL PARAMETERS FOR FOOD SAFETY MANAGEMENT

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**Objectives:** The purpose of this study was to develop product microbial baselines at various locations throughout a commercial poultry processing facility operating under the New Poultry Inspection System (NPIS) under high vs. low chemical intervention schemes to establish statistical process control thresholds for food safety management and regulatory compliance.

**Materials and Methods:** The experimental design consisted of a completely randomized design with chemical concentration level as a fixed effect at each stage of the poultry processing chain. Treatments consisted of antimicrobial concentration usually ran by the processing plant (CX) (100-400 ppm of peroxyacetic acid and 50 ppm of total chlorine) and reduced chemical concentration (RC). Nine locations throughout the processing line were sampled; including: live receiving (LR), rehanger (RH), post evisceration (M), post-Cropper (C), post-neck breaker (NB), inside-outside bird wash one (IOBW1), inside-outside bird wash two (IOBW2), pre-chill (PRE), post-chill (PC), and wing parts (WINGS). At each location, ten rinsate samples were taken per repetition for CX and RC treatments, five per shift. A total of 810 samples were taken during a seven-month period. Aerobic (AC) and *Enterobacteriaceae* (EB) counts were determined by the Tempo<sup>®</sup> system following manufacturer's instructions. *Salmonella* counts and prevalence were determined using SalQuant<sup>TM</sup> and BAX<sup>®</sup> system Real-Time *Salmonella* assays, respectively. All counts were transformed to LogCFU/mL of rinse and statistical analysis was conducted to determinate differences between CX and RC treatment at each location with 0.05 probability threshold using RStudio, version 4.04. When parametric assumptions were not met, the wilcoxon rank sum test was performed.

**Results:** Aerobic Counts were not different (P > 0.05) between CX and RC treatments at RH, M and C locations. Counts in NB, IOBW1, IOBW2, PRE, PC and WINGS showed a difference between treatments with a mean difference of 0.39 Log CFU/ml, where reduced chemical conditions show greater counts. For EB counts, M counts had significant difference between treatments with a mean difference of 0.85 Log CFU /ml, with higher concentration in CX. On average, RC treatment led to higher counts at NB, IOBW1, IOBW2, PRE and WINGS locations. No difference (P > 0.05) were found in RH, C and PC locations. In this study, 200 of 810 samples were suitable for enumeration using the BAX<sup>®</sup> System SalQuant<sup>™</sup>, the majority found at LR. *Salmonella* counts were different (P < 0.05) in all sampling points. The RC treatment led to greater counts with an average difference of 0.37 Log CFU/ml.

**Conclusion:** Pathogen quantification can result in appropriate risk assessment where chemical intervention can be targeted to stages with higher pathogen concentrations. This study provides evidence for the application of reduced chemical schemes in different stages of processing, tailoring interventions for higher risk areas. The development of biomapping baselines will result in statistical process control analysis to support food safety management decision-making. Nonparametric statistical process control can be approached to more representatively use pathogen prevalence and quantification data together, resulting in more educated decisions than using solely prevalence data.

Keywords: Antimicrobial interventions, Bio-Mapping, NPIS, Salmonella Quantification, Statistical Process Control



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### QUANTIFICATION OF SALMONELLA AND INDICATOR MICROORGANISMS FOR BIOMAPPING AT DIFFERENT STAGES IN A PORK PROCESSING FACILITY OPERATING UNDER NEW SWINE INSPECTION AND FEATURING A BIO-FURNACE CABINET.

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Objectives: The purpose of this study was to develop a quantitative baseline of indicator microorganisms and Salmonella by bio-mapping microbial changes through a pork production process with a Bio-Furnace cabinet. Materials and Methods: A 10-week study was conducted to collect five samples at each of 11 locations throughout a commercial pork processing facility (n = 550). Sampling locations included: post-scalding (PS), after bio-furnace (AF), before final rinse (BR), after final rinse (AR), post-chilling (PC), and on the following final products; boneless picnic (BP), belly trim (BT), butt trim (BTT), loin trim (LT), and advanced meat recovery product (AMR). Swab samples were taken at the indicated harvest locations while 2-pound samples were collected for trim and ground product. Samples were collected using the protocols for Whole Pork Cuts and Comminuted Pork Aseptic Grab Sample Not in Final Packaging. Swab and product samples were immediately chilled and shipped overnight. Swab and trim samples were prepared utilizing BAX® MP for indicators (AC, Enterobacteriaceae, and generic E. coli). The samples were analyzed for indicator enumeration with the BioMerieux Tempo<sup>®</sup>. Salmonella prevalence and enumeration was evaluated using the BAX<sup>®</sup> System Real-Time Salmonella and the SalQuant<sup>™</sup> methodology. Microbial counts were converted to Log10CFU/sample. **Results:** Total APC were reduced from 4.4 Log<sub>10</sub> CFU/ sample on carcasses PS, to less than 2.8 Log<sub>10</sub> CFU/ sample PC. APC counts ranged from 2.1 to 3.1 Log<sub>10</sub> CFU/ sample on trim, with AMR having the highest count at 3.3 Log<sub>10</sub> CFU/ sample. Results for both EB and EC were relatively low. EB and EC were detectable in select sampling locations, while APC was countable at all locations. EB counts were found the highest on the PS samples and none were quantifiable after post chilling. BT, BTT, and LT along with AMR all had approximately 1-2 Log<sub>10</sub> CFU/ sample of EB post chilling. EC was quantifiable on PS samples, but not at any other point in the process after the Bio-Furnace Cabinet. Salmonella counts followed a similar pattern as EB. On the harvest floor, Salmonella was present at approximately 2 logs at all points until PC samples. After chilling, Salmonella was not quantifiable on the carcass. However, there was some quantifiable Salmonella at 1.8 - 4.1 Log<sub>10</sub> CFU/sample present on AMR and BT, respectively, after chilling thus indicating the need for interventions during further processing.

**Conclusion:** A quantitative microbial biomap was developed in a large USDA-inspected pork processing plant after the implementation of the New Swine Inspection System (NSIS). This study shows that indicators and *Salmonella* levels were reduced through the process and not detected after chilling, demonstrating significant control with final reductions occurring in the bio-furnace in this study. However, in trim and AMR samples, both *Salmonella* and indicators were detected and quantifiable at certain points indicating the need for interventions after carcass processing. The use of a rapid PCR-based enumerative method for pathogens, in conjunction with indicator levels, provides the pork industry with a tool for data-driven decisions to target points of concern in the process, establish statistical control thresholds, and mitigate the risk to public health of foodborne illness.

Keywords: Microbial Indicators, New Swine Inspection System, Pork Processing Plant, Salmonella



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### QUANTITATIVE BIO-MAPPING OF SALMONELLA AND INDICATOR ORGANISMS THROUGHOUT A PORK PROCESSING LINE TO ESTABLISH STATISTICAL PROCESS CONTROL PARAMETERS FOR FOOD SAFETY MANAGEMENT

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**Objectives:** The purpose of this study was to develop a baseline based on quantification of indicator organisms and *Salmonella* by bio-mapping through the processing chain from harvest to final product within a commercial pork processing establishment to demonstrate microbial control in pork processing operations implementing the New Swine Inspection System (NSIS).

**Materials and Methods:** Five individual samples, including harvest swabs, pork trim and ground pork; were collected at 13 sampling locations (Harvest: Gambrel Table, After Polisher, Before Final Rinse, After Final Rinse and Post Snap Chill, After Peracetic (PAA) Cabinet; Trim: Boneless Picnic, Belly Trim, Neck Trim and Loin Trim; Further Process: Advanced Meat Recovery (AMR), Ground Brick Trim and Sausage Links), over a 10-week period (n=650). Swabs were collected on the processing line; trim and further process samples consisting of two pounds were immediately chilled and shipped overnight to the ICFIE Food Microbiology Laboratory at Texas Tech University. All samples, except ground products, were prepared utilizing a single enrichment source (BAX<sup>®</sup> MP) for indicator organisms (Aerobic

Count, Enterobacteriaceae, and E. coli). Indicators were enumerated using the bioMérieux

TEMPO<sup>®</sup>. Salmonella prevalence and enumeration were determined with the BAX<sup>®</sup> System Real-Time Salmonella and SalQuant<sup>™</sup>, respectively. Microbial counts were converted to Log<sub>10</sub>CFU/ml(g) and Log<sub>10</sub>CFU/sample prior to statistical analysis.

**Results:** All indicator microorganisms were significantly reduced at the harvest floor (P<0.001), from Gambrel Table to After PAA Cabinet. The reduction at Harvest was 2.27 Log<sub>10</sub>CFU/ml for aerobic counts (AC), 2.46 Log<sub>10</sub>CFU/ml for *Enterobacteriaceae* (EB) counts, and 2.24 Log<sub>10</sub>CFU/ml for *E. coli* (EC) counts. Trim sample values fluctuated based on cut, with the highest AC count found at Neck Trim (2.83 Log<sub>10</sub>CFU/g). Further process samples showed the highest AC count in Sausage with a mean of 5.28 Log<sub>10</sub>CFU/g. EB counts in Sausage (3.19 Log<sub>10</sub>CFU/g) were increased, compared to the reduction observed at the end of harvest and throughout trim processing. EC counts showed a similar trend to EB counts with the highest value found in Sausage Links (1.60 Log<sub>10</sub>CFU/g). Statistical microbial process control parameters were also developed for each of the indicator microorganisms, using the sample mean ( $\overline{X}$ ), the Lower control limit (LCL) and Upper control limit (UCL) with ±3 $\sigma$  at each sampling location. For *Salmonella* prevalence, a total of 126/650 samples were found positive (19%). From those positive samples, 52 samples (41%) were suitable for enumeration using the BAX<sup>®</sup> System SalQuant<sup>TM</sup>, the majority detected at the Gambrel Table location. The *Salmonella* counts at Gambrel Table, After Polisher, Before Final Rinse and After Final Rinse locations ranged from <1 CFU (Limit of Enumeration) to 4.02 Log<sub>10</sub>CFU/sample. There was an average *Salmonella* reduction of 1.87 Log<sub>10</sub>CFU/sample on the harvest floor. For trim samples, *Salmonella* counts were highest for Boneless Picnic (2.87 Log<sub>10</sub>CFU/sample).

**Conclusion:** This study provides evidence for the application of emerging technologies for pathogen quantification and indicator levels in pork samples. It provides the basis for developing statistical process control variables based on process bio-mapping baselines at different stages during processing to support food safety management decision-making for controlling pathogens in pork products and guide process changes and speed line modifications.

Keywords: microbial indicators, New Swine Inspection System, pork processing plant, Salmonella



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### LYTIC BACTERIOPHAGE AND LACTIC ACID AGAINST E. COLI O157:H7 IN MARINATED AND TENDERIZED PORK LOINS

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**Objectives:** It is evident that pork is an underrecognized source of Shiga Toxin-producing *Escherichia coli* (STEC), however, there are multiple studies and documented cases providing evidence for the pork-related STEC O157:H7 infections. Thus, *E. coli* O157:H7 is becoming an increasing concern for the pork industry despite several attempts to eliminate the microorganisms in several critical steps.

This study aims to evaluate the effect of lytic bacteriophages (phage) and lactic acid (LA) as antimicrobials in reducing the presence of *E. coli* O157:H7 on the surface and internal cores of marinated and tenderized pork loins.

**Materials and Methods:** *E. coli* O157:H7 from three different sources (hamburger - NFPA 4200, salami - NFPA 4212, lettuce - NFPA 4217) were maintained on Tryptic Soy Agar throughout the experiment. Locally purchased raw pork loins were sliced into 2" - 2½" thick chops (n=30/replication). Pork chops were inoculated with a *E. coli* O157:H7 cocktail with a concentration of 10<sup>6</sup> CFU/ml for 30 min attachment at 4°C. Inoculated chops were marinated for one hour with 0.35% sodium chloride and 0.45% sodium tripolyphosphate. Marinated pork chops were randomly divided into one of the five different treatments (Control, DI water, LA 2.5%, phage 5%, LA 2.5% + phage 5%) and were tenderized through a manual meat tenderizer. Treatments were applied  $(10\mul/cm^2)$  to cover the entire meat surface area. Surface swabs (50 cm<sup>2</sup>) were taken before and after the meat tenderization process. Sterile internal meat cores were homogenized and evaluated for the possible pathogen translocation by plating on CT-SMAC agar. Response variables of interest include surface pathogen attachment, internal pathogen concentration, and meat pH. Pathogen cell count was transferred to  $log_{10}$  CFU/ cm<sup>2</sup> or g prior to the statistical analysis. PROC MIXED procedure of SAS was used as a statistical tool to analyze. Mean separation was done using the DIFF function, which is used to determine significant differences (*P* < 0.05) among means using ANOVA.

**Results:** We hypothesize that the application of LA 2.5% and lytic bacteriophage 5% on marinated pork chops will result in the significant reduction of *E. coli* O157:H7 counts on the surface, and internal cores, whereas the combined treatment will result in an even higher log reduction of the pathogens. With the first replication in hand, the data indicate varied antimicrobial susceptibility patterns of *E. coli* cocktails in marinated pork loins. Marinated loins treated with phage and LA + phage significantly reduced the surface pathogens after 60 minutes of cold storage when compared with control. Similarly, phage-treated loins showed a significant reduction of more than 2.40 logs of *E. coli* (P < 0.05) on the post-tenderized loin surface. However, no difference was observed (P > 0.05) for the post-tenderized surface counts between DI, LA and LA + phage treated loins. Likewise, no difference (P > 0.05) was observed between phage, DI and LA treated loins for the number of translocated pathogens from the surface to the internal core after manual tenderization. **Conclusion:** *E. coli* 0157:H7 – specific lytic bacteriophage and LA reduced the number of surface pathogens translocated from the surface to the internal cores and on post-tenderization surface counts for marinated pork loins (60 min).

Keywords: E. coli O157:H7, Food Safety, Lactic Acid, Lytic Bacteriophage, Pork



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### APPLICATION OF PREDICTIVE MODELING IN THE VALIDATION OF A HOME-STYLE RESTRUCTURED BEEF JERKY PRODUCT DESIGNED FOR PRODUCTION IN ETHIOPIA

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**Objectives:** Ethiopia is home to the tenth largest livestock inventory in the world. Despite the availability of livestock, the population still has one of the lowest per capita red meat consumptions in the world. Inefficiencies in the meat supply chain have limited the access to animal sourced food and demonstrate the need for additional preservation techniques to improve the accessibility of meat. Drying is one of the oldest food preservation methods, involving the addition of salt and removal of water to make a shelf-stable, nutrient dense, and ideally "safe" product. Pathogens have demonstrated the ability to adapt during a slow drying process, making them more resistant to later heat treatments and complicating process validation. The objective of this study was to quantitatively evaluate the inactivation kinetics of foodborne pathogens during the drying of a restructured beef jerky product in a home-style dehydrator.

**Materials and Methods:** Challenge studies were performed to evaluate the inactivation of five serotypes of *Salmonella enterica* (Saintpaul, Anatum, Typhimurium, Newport, Dublin), three strains of *Escherichia coli* O157:H7, and three strains of *Campylobacter jejuni*. Berbere, an Ethiopian spice mixture, and salt were added to lean ground beef. Each treatment was inoculated with a cocktail of isolates, formed into strips, and dehydrated for 6 h in a home-style dehydrator (600W). Samples were weighed pre- and post-drying, plated for enumeration at times 0, 1, 2, 3, 4, 5, and 6 h, and water activity (a<sub>w</sub>) was measured at each sampling interval.

**Results:** The dry-bulb temperature steadily increased for the first 3 h of drying before stabilizing at  $61.8 \pm 0.6$ °C and the relative humidity stabilized at approximately 10%. Non-linear predictive models were fitted to the inactivation data revealing an inverse sigmoidal curve for the inactivation of Salmonella and concave downward curves for the inactivation of E. coli O157:H7 and *Campylobacter*. The predictive models suggest that after 6 h of drying a 4.56- and 6.27-log (CFU/g) reduction of *Salmonella* and *E. coli* O157:H7 will be achieved, and after 3 h of drying a 4.32-log (CFU/g) reduction of *Campylobacter* will be achieved. The shape of these curves suggests the need for a higher wattage dehydrator to either shorten the come-up time, or increase the maximum temperature, or both to reduce the opportunity for Salmonella to desiccate before thermal lethality.

**Conclusion:** Additional processes to extend the shelf life of animal sourced foods in Ethiopia will provide consumers with a more convenient and accessible source of protein, as well as provide producers with a secondary avenue to market their product. Predictive models of pathogen inactivation allow producers, in Ethiopia and the United States, to more accurately assess the microbial risk of their product at any point during production.

Keywords: Campylobacter, E coli, Inactivation kinetics, Salmonella, Water activity



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### BIOMAPPING OF MICROBIAL INDICATORS ON BEEF CARCASSES, SUBPRIMALS, AND TRIMMINGS THROUGH THE PROCESSING LINE AFTER APPLICATION OF MULTIPLE ANTIMICROBIAL INTERVENTIONS

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**Objectives:** To determine load reductions of indicator microorganisms after application of antimicrobial interventions through the beef processing line.

Materials and Methods: EZ-Reach<sup>™</sup> swabs pre-hydrated with 25 ml buffered peptone water were used to collect beef samples before and after intervention treatments on a 100 cm<sup>2</sup> area in the foreshank area of the carcass at 4 processing stages in a commercial operation: hot carcass just before entering the hot box (n=540), cold carcass after 24 h of chilling (n=300), subprimals just before packaging (n=972), and beef trimmings coming off of the fabrication floor (n=540). A total of 6 subprimals, brisket, shoulder clods, top butts, knuckles, loin tails, and chuck rolls, as well as 3 types of trim, shank, chuck, heel were evaluated. Unpaired sampling was conducted at every processing stage. Hot carcass intervention consisted of hot water wash with a water temperature of 76-87°C followed by a lactic acid spray with a temperature of 43-55°C at 2-5% lactic acid concentration at a pressure  $\geq$  15 psi. Cold carcass intervention consisted of a lactic acid spray with solution at 43-55°C and 2-5% lactic acid concentration at ≥ 15 psi. Subprimal intervention consisted of lactic acid spray with a temperature of 43-55°C at 2-5% lactic acid concentration with a pressure of  $\geq$  15 psi. Trim intervention consisted of lactic acid trim submersion with a solution at 43-55°C and 2-5% concentration of lactic acid. Swabs were immediately chilled and shipped overnight to the ICFIE Food Microbiology Laboratory at Texas Tech University. Samples were homogenized in a stomacher at 230 rpm for 1 min, serially diluted and plated onto 3M<sup>™</sup> Petrifilm<sup>™</sup> plates to determine total aerobic plate counts (APC), coliform (CC), and generic *E. coli* (EC) counts. APC petrifilm were incubated for 48  $\pm$  3 h at 35  $\pm$  1 °C. The CC/EC petrifilms were incubated for 48  $\pm$  3 h at 35  $\pm$  1 °C. CC counts were determined after 24-hour incubation. EC counts were determined after 48h incubation. Microbial counts converted to LogCFU/cm<sup>2</sup> before statistical analysis.

**Results:** All indicator microorganisms were significantly reduced at each stage after each intervention (*P*<0.05). APC counts were reduced by 4.59 LogCFU/cm<sup>2</sup> in hot carcass, 0.68 LogCFU/cm<sup>2</sup> in cold carcass, and by at least 0.37 LogCFU/cm<sup>2</sup> in the evaluated subprimals and 0.84 LogCFU/cm<sup>2</sup> in analyzed trim samples. CC counts were reduced by 4.20 LogCFU/sample in hot carcass 0.74 LogCFU/sample in cold carcass, by at least 0.97 LogCFU/sample in the evaluated subprimals and 0.81 LogCFU/sample in analyzed trim samples. EC counts were reduced by 4.10 LogCFU/sample in hot carcass, 0.15 LogCFU/sample in cold carcass, and by at least 1.01 LogCFU/sample in the evaluated subprimals and 0.65 LogCFU/sample in analyzed trim samples. Final product average counts were 0.61 LogCFU/cm<sup>2</sup>, 0.60 LogCFU/sample and 0.32 LogCFU/sample for APC, CC, and EC counts respectively.

**Conclusion:** Additional interventions in post chill stages are effective in further reducing indicator microorganism counts. A multiple hurdle approach of applying different interventions can continuously reduce indicator microorganisms through the beef processing chain. The interventions applied at every step effectively reduced indicator microorganisms, thus potentially improving the safety of the final product.

Keywords: antimicrobial, Beef processing plant, Lactic Acid, microbial indicators



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### NOVEL OZONE GENERATION TECHNOLOGY (BIO-SAFE) INTERVENTION COMPARED TO LACTIC ACID IN VARIETY MEATS ON A BEEF PROCESSING PLANT

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**Objectives:** The purpose of this study was to compare the antimicrobial efficacy of an aqueous ozone intervention and a lactic acid solution on natural microbiota of variety meats in a commercial beef processing plant.

**Materials and Methods:** EZ-Reach<sup>™</sup> swabs pre-hydrated with 25 ml of buffered peptone water were used to collect 500 cm<sup>2</sup> area samples before and after ozone and lactic acid intervention application for three different variety meats (head, heart, and liver). Lactic acid intervention parameters included a spray treatment with a temperature of 43-55 °C at 2-4 % lactic acid concentration and spray pressure ≥ 15 psi. The aqueous ozone intervention spray had a concentration 1.5-2.3 ppm, an oxidation-reduction potential (ORP) between 700 and 900 mV with a spray pressure ≥ 20 psi, and an incoming water maintained at 10-24 °C. The ozone intervention consisted of one cabinet with 44 nozzles delivering 12.8 gpm with 18 s contact time. Each repetition included 54 samples per variety meat and antimicrobial, 27 before and 27 after intervention, for a total of 162 samples per repetition. Swab samples were immediately chilled and shipped overnight to the ICFIE Food Microbiology Laboratory at Texas Tech University for microbial analysis. Samples were stomached at 230 rpm for one minute and for each variety meat, 3 individual samples were composited into one. Serial dilutions and enumeration of total aerobic bacteria (APC) and *Escherichia coli* were performed on each composite. Counts were transformed into Log CFU/cm<sup>2</sup> and statistical analysis was conducted to determine differences between before and after intervention with 0.05 probability threshold. A total of 6 repetitions were conducted throughout the whole study, where the lactic acid treatment occurred one full year before plant modifications using the ozone delivery system. This study compares microbial performance year-to-year differences under modified antimicrobial schemes.

**Results:** APC and *Escherichia coli* counts were transformed to Log CFU/sample for statistical analysis as counts were considerably low when analyzed on a Log CFU/cm<sup>2</sup> basis (detection limit =  $0.002 \text{ CFU/cm}^2$ ). Microbial counts for both microorganisms evaluated were significantly reduced (P < 0.001) after lactic acid immersion (2-5%) and ozone intervention for all variety meats with exception of ozone intervention in *Escherichia coli* counts in heart samples. Aerobic plate counts after lactic acid intervention were reduced on average by 1.73, 1.66, and 1.50 Log CFU/sample in head, heart, and liver, respectively. Samples collected under the ozone intervention scheme, showed that counts were reduced on average by 1.66, 0.52 and 1.20 Log CFU/sample. *Escherichia coli* counts after lactic acid intervention were reduced on average by 0.96, 0.79, and 1.00 Log CFU/sample in head, heart, and liver, respectively, while after ozone intervention, counts were reduced on average by 0.75, 0.62 and 1.25 Log CFU/sample.

**Conclusion:** The aqueous ozone antimicrobial scheme proved to be promising as an intervention for the reduction of indicator levels in variety meats. These findings suggest that bio-safe ozone intervention may play a competitive role as lactic acid applications on reducing bacterial load on variety meats, thus contributing to food safety control.

Keywords: beef variety meats, Lactic Acid, microbial indicators, ozone intervention



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### STORAGE TEMPERATURE AND DURATION IMPACT SPOILAGE ORGANISM GROWTH IN VACUUM PACKAGED BEEF STRIP LOINS

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**Objectives:** The objective of this study was to evaluate the influence of storage temperature, duration, and their interaction on spoilage organism growth.

**Materials and Methods:** Paired USDA Choice strip loins (IMPS #189; n=60) were collected from a commercial beef processing facility. Each carcass was assigned to a storage temperature (-2, 0, 4°C). Strip loins were portioned into half loins and assigned to an aging duration (14, 28, 42, 56d). Loins were aged in commercial upright refrigerators and temperatures were monitored with data loggers. At each aging interval, the vacuum packaging was opened to expose the cut surface of the longissimus lumborum. The cut surface was swabbed using pre-hydrated buffered peptone water (BPW) swabs and a 50 cm<sup>2</sup> template. Lactic acid bacteria (LAB; mesophilic), *Enterobacteriaceae* (EB; mesophilic), and aerobic plate counts (M-APC, P-APC; mesophilic, psychrotrophic) were spoilage organisms of interest. LAB, EB, and M-APC were enumerated using the TEMPO System. P-APC were enumerated, in duplicate, on APC Petrifilms. Serial dilutions were performed using BPW tubes, as necessary. Data were log<sub>10</sub> transformed for statistical analysis. The detectable limit was 0.02 CFU/cm<sup>2</sup>. Data were analyzed as a split-plot where storage temperature served as the whole plot and aging duration served as the subplot. An alpha of *P* <0.05 to determine signifcance.

**Results:** Storage temperature × aging duration interactions were observed for all spoilage organisms (P <0.001). Loins aged for 42 d at 4°C had the greatest EB counts compared to all other treatments (P <0.001). EB counts in loins aged for 28 and 56 d at 4°C were similar (P>0.05). Within the 4°C environments, loins aged for 14 d possessed the lowest EB counts. EB growth was similar between loins aged at –2 and 0°C regardless of duration (P >0.05). M-APC growth was the lowest in loins aged for 14 d at -2°C (P<0.001). Loins aged for 28d and 42 d at either 0 or 4°C possessed increased M-APC growth (P<0.001). Similar to M-APC, LAB growth was lowest in loins aged for 14 d at -2 or 0°C (P<0.001). Loins aged for 28, 42, and 56 d at 4°C and 42 d at 0°C possessed the greatest LAB microbial loads (P<0.001). P-APC growth was greater in loins aged at 4°C compared to loins aged in -2 or 0°C (P<0.001). Loins aged for 14 d at 4°C were similar to loins aged for 20 or 56 d at -2°C (P<0.001).

**Conclusion:** These data indicate that storage temperature influences the growth of spoilage organisms at different aging durations. Aging beef subprimals in colder environments mitigates spoilage organisms growth during extended aging.

Keywords: beef, shelf life, spoilage organisms, temperature, wet aging



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### TANDEM MASS TAG LABELING-BASED ANALYSIS OF BISON MUSCLES TO CHARACTERIZE MUSCLE-SPECIFIC PROTEOME CHANGES DURING AGING

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**Objectives:** The objective of the study was to examine the variations in sarcoplasmic proteome profile in bison *longissimus lumborum* (LL; colour-stable) and *psoas major* (PM; colour-labile) muscles during postmortem aging periods (2 d, 7 d and 14 d) using tandem mass tag (TMT) isobaric labeling coupled with liquid chromatography mass-spectrometry (LC-MS) for the segregation of muscles with muscle-specific inherent colour stability.

**Materials and Methods:** Six (n = 6) *longissimus lumborum* (striploins) and six (n = 6) psoas *major* (tenderloins) muscles from A1 grade bison carcasses were collected from a federally inspected slaughter plant within 48 h postmortem. Each muscle portion was sub-sampled (15-20 g) for the sarcoplasmic proteomic analysis at 2 d postmortem (baseline) and vacuum packaged and stored immediately at -40 °C. The remaining muscle portions were equally divided into two parts and stored at 2 °C for an aging period of 7 and 14 d. At the end of each aging period, sample for proteomic analysis was collected from each muscle, vacuum packaged and stored at -40 °C for subsequent analysis. TMT labeling based proteomic analysis was performed coupled with LC-MS/MS. After collecting the MS data, tryptic peptides were searched and identified against the bison protein database using X! Tandem software.

**Results:** A total of 576 proteins were identified in both bison LL and PM muscles where 96 proteins were found differentially abundant (fold change > 1.5, P < 0.05) from the three comparisons between muscles during postmortem storage periods (PM vs LL at 2 d, 7 d and 14 d). Among those proteins, the most important identified protein groups based on function are related to electron transport chain (ETC), tricarboxylic acid cycle (TCA), ATP production and transport, carbohydrate metabolism (mostly glycolytic enzymes), lipid or fatty acid degradation, chaperones, oxygen transport, calcium signaling, muscle contraction and protein synthesis. In LL muscles, the dehydrogenase proteins of electron transport chain (ETC), tricarboxylic acid cycle (TCA), fatty acid (FA) oxidation, carbohydrate metabolism, and ATP transport proteins were overexpressed or upregulated during aging than PM. On the other hand, the proteins of carbohydrate metabolism (glycogen phosphorylase, phosphoglucomutase-1, adenylate kinase isoenzyme-1) related to ATP utilization were downregulated in LL muscles compared to PM. During comparisons of PM vs LL muscles, protein-protein interactions showed specific networks at 2 d (ETC, FA oxidation and muscle contraction), 7 d (muscle contraction) and 14 d (carbohydrate metabolism, FA oxidation and muscle contraction). The results clearly indicate that the dehydrogenase enzymes responsible for reducing equivalents and ATP production were highly expressed in LL muscles resulting oxidative stability and subsequent meat color stability. Moreover, the glycolytic proteins involved in ATP utilization were downregulated in LL muscles reflecting more colour stability.

**Conclusion:** The TMT-based proteomic analysis showed a clear segregation of two bison major muscles where PM showed lower color stability compared to LL. To the best of our knowledge, this is the first attempt to compare the proteomic profile of bison LL and PM muscles from different storage periods. Therefore, this isobaric tag-based analysis was able to uncover the proteomic profile of bison muscles with inherent color stability.

**Keywords**: Color stability, segregation, proteomics, sarcoplasmic proteins, isobaric tags, liquid chromatography mass-spectrometry



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MYOGLOBIN POST-TRANSLATIONAL MODIFICATIONS INFLUENCE FRESH BEEF COLOR STABILITY

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Objectives: Post-translational modifications (PTM) play a fundamental role in regulating biological processes. The complex relationship between PTM of muscle proteins and meat quality has become increasingly evident recently. Nonetheless, investigations are yet to be undertaken to characterize myoglobin PTM and their role in fresh meat color stability. Therefore, the objectives of the current study were to identify the sites of myoglobin PTM in post-mortem beef longissimus lumborum (LL) muscle during aging and to evaluate their influence on fresh beef color stability. **Materials and Methods:** Beef LL muscles collected from right side of nine (n = 9) beef carcasses (USDA choice; A maturity; 24 h post-mortem aging) were divided into 4 equal-length sections. The muscle sections were vacuum packaged and randomly assigned to wet aging at 2°C for either 0, 7, 14 or 21 days. At the end of each wet-aging period, the muscle sections were removed from the vacuum package and fabricated into four 1.92-cm thick steaks. One steak from each muscle section allotted for proteome analyses was immediately vacuum packaged and frozen at -80°C until used. The remaining three steaks assigned for evaluation of instrumental color and biochemical evaluation were aerobically packaged and assigned to refrigerated storage (2°C) in the darkness for either 0, 3 or 6 days. Myoglobin PTM were analyzed using two-dimensional electrophoresis and tandem mass spectrometry. The instrumental color and biochemical properties were analyzed as a split-plot design with aging time as a whole-plot factor, and storage day as a sub-plot factor. Carcass was considered as random effect. The data were analyzed using PROC MIXED procedure in SAS, and the differences among the means were detected using the least significant differences at a P < 0.05 level. **Results:** Aging influenced fresh beef color attributes; surface redness (a\* value), color stability (R630/580), and myoglobin concentration decreased (P < 0.05) upon aging. Image analysis of two-dimensional gels identified six spots as myoglobin, with similar molecular weight (17 kDa) but different isoelectric pH, indicating that myoglobin was posttranslationally modified. Tandem mass spectrometry identified multiple PTM (phosphorylation, methylation, carboxymethylation, acetylation, and hydroxynonenal alkylation) in the six myoglobin spots. The amino acid residues susceptible to phosphorylation were identified as serine, threonine and tyrosine, whereas methylation, acetylation and carboxymethylation were detected in lysine and arginine residues. Additionally, distal histidine (histidine 64) which is critical to heme stability was readily modified by hydroxynonenal alkylation. Furthermore, myoglobin PTM sites increased with aging time from day 0 to day 14, whereas decreased afterwards. The decrease in number of PTM from day 14 to day 21 was possibly due to the decrease in myoglobin concentration.

**Conclusion:** Myoglobin in beef LL muscle undergoes PTM during aging. These PTM compromise myoglobin redox stability by adding modifying groups to amino acid residues—especially those close to the hydrophobic heme pocket— and therefore accelerate myoglobin oxidation and beef discoloration. These *in situ* myoglobin PTM could be utilized as novel biomarkers for color stability in muscle foods.

Keywords: Meat color, Myoglobin, Post-translational modifications



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### HIGH PULMONARY ARTERIAL PRESSURE IN STEERS AT MODERATE ALTITUDE AFFECTS EARLY POSTMORTEM MITOCHONDRIA FUNCTIONALITY

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**Objectives:** Pulmonary hypertension is a noninfectious disease of cattle at altitudes > 1524 m (5,000 ft). Mean pulmonary arterial pressures (PAP) is used as an indicator for pulmonary hypertension in cattle. High PAP cattle (≥ 50 mmHg) entering the feedlot at moderate elevations have lower feed efficiency as compared to low PAP cattle (< 50 mmHg). The *longissimus lumborum* (LL) steaks from high PAP steers have lower color and lipid stability than those from low PAP steers during retail display. Mitochondria can affect animal production efficiency, and their functionality in postmortem muscle contributes to meat color and tenderness development. However, the effect of PAP on muscle mitochondria in beef cattle has not been investigated. Therefore, the objective of this study was to examine the effect of PAP on beef mitochondrial function during the early postmortem period.

**Materials and Methods:** Mitochondrial function and oxidative phosphorylation (OXPHOS) protein abundance of *longissimus lumborum* (LL) muscle from high PAP (98±13; n = 5) and low PAP (41±3; n = 6) fattened Angus steers (i.e., live weight of ~600 kg) were evaluated during early postmortem period (2 h and 48 h postmortem) by a split-plot design. Data analysis was performed using R with the lme4 package as a mixed model, where PAP score (high or low), postmortem time, and their interactions were the fixed effects, and random effect was individual steer. The differences between least-square means (P < 0.05) were determined by Tukey's multiple comparison.

**Results:** A PAP x postmortem-time interaction (P < 0.05) was observed in the proton leak-associated respiration (supported by high NADH) and OXPHOS-linked-respiration (supported by pyruvate + malate). This suggested that high PAP muscle tended to have a greater respiratory capacity at 2 h postmortem and a faster decline in mitochondrial respiratory capacity from 2 h to 48 h postmortem than low PAP muscle. In addition, there was a decline (P < 0.05) in OXPHOS-linked respiratory capacities (supported by maximal delivery of NADH and NADH + succinate), as well as the oxygen reductase capacity of complex IV in LL muscle from 2 h to 48 h postmortem, which suggested that the maximal respiratory capacity of LL declined after animal slaughter independent of PAP. Further, muscle OXPHOS protein expression was greater in low PAP than high PAP animals (P < 0.05) regardless of postmortem time, which suggested a greater mitochondria content in low PAP muscle and a potential shift in muscle fiber type of the LL muscle. **Conclusion:** High PAP caused a lower OXPHOS efficiency and a greater fuel oxidation rates under conditions of low ATP demand in beef LL muscle. This could explain the lower feed efficiency in high PAP feedlot cattle compared to low PAP counterparts. Mitochondrial integral function declined faster in high PAP compared to low PAP muscle at early postmortem, which could be leading to the lower color and lipid stability of high PAP LL during retail display. Pulmonary arterial hypertension could also decrease type I/type II muscle fiber ratio in skeletal muscle, which needs to be investigated further.

Keywords: longissimus lumborum, mitochondrial function, pulmonary hypertension



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### AN INVESTIGATION OF THE RELATIONSHIP BETWEEN SARCOMERE LENGTH AND MEAT TENDERNESS AND A NOVEL WAY TO MEASURE SARCOMERE LENGTH.

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**Objectives:** Sarcomere length (SL) has long been considered as an indicator for meat tenderness because it determines the level of muscle contraction. Historically, SL is commonly measured by the laser diffraction method proposed by Cross et al., 1981. While this method is widely accepted, it is labor intensive and could potentially result in serious damage in eyesight; furthermore, it also lacks tangible proof of actual measurement of SL. Therefore, the objective of the study was to develop a novel and convenient way to measure SL by immunostaining powdered meat, as well as to investigate the relationship between Warner-Bratzler Shear Force (WBSF) and SL of 8 different beef muscles with two aging periods. **Materials and Methods:** Eight muscles were collected from 10 USDA choice beef carcasses: supraspinatus (SS), longissimus thoracis (LT), rectus femoris (RF), rectus abdominis (RA), triceps brachii (TB), semitendinosus (ST), gluteus medius (GM), and pectoralis profundus (PP). The muscles were cut into steaks and assigned to a 2 or 21 day aging period (n = 160). Each steak was pulverized in liquid nitrogen prior to analysis. A few meat speckles from each sample were placed on a microscope slide and incubated with anti- $\alpha$ -actinin as a primary antibody, followed by incubating with Alexa-Flour Plus 488 as the secondary antibody. Finally, the slide was imaged using an upright confocal microscope with a 63x/1.4 oil objective. A total of 30 sarcomeres were measured and averaged for each sample. Additionally, each sample's tenderness was measured by WBSF method. A correlation analysis was performed to elucidate the relationship between SL and WBSF.

**Results:** There was no interaction between muscle and aging time for SL (P > 0.10), but a main effect was found for muscle (P < 0.05). PP (2.47 µm) and RA (2.46 µm) had the longest SL, followed by SS (2.24 µm), TB (2.09 µm), RF (2.01 µm), and ST (1.88 µm), with LT (1.75 µm) and GM (1.65 µm) having the shortest sarcomeres among all (P < 0.01). In addition, PP (6.93 kg) had the highest WBSF, followed by SS (5.37 kg), ST (5.27 kg), GM (4.62 kg), RA (4.53 kg), TB (4.17 kg), RF (3.97 kg), and finally LT (3.72 kg) with the lowest WBSF among all (P < 0.01). A positive correlation coefficient (r) of was only found between SL and WBSF for SS (r = 0.50; P < 0.05), but not for any other muscle utilized in this study. Finally, an overall positive relationship between SL and WBSF was found for all muscles combined and measured as a whole (r = 0.39, P < 0.01).

**Conclusion:** Measuring SL by staining  $\alpha$ -actinin is feasible, and it yields comparable results to other studies that reported SL on these same muscles. It is interesting to point out that tougher muscles tended to have longer sarcomeres. However, no clear relation was found between SL and WBSF on an individual muscle basis.

Keywords: beef, sacromere lenght, tenderness, WBSF



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### VOLATILE COMPOUNDS AND AMINO ACID CONTENT ARE CORRELATED WITH OFF-FLAVOR INTENSITY OF DRY AND WET-AGED USDA PRIME AND CHOICE STRIP LOINS AGED FOR 21 AND 42 DAYS.

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**Objectives:** Dry-aging beef has been associated to a process that enhance eating experience by improving flavor. Flavor perception depends on a multisensory integration led by chemical compounds that are detected via olfactory-gustatory interactions. Amino acids are part of a large pool of reactive components that may directly or indirectly affect flavor by reacting with reducing sugars to form Maillard reaction products and Strecker degradation products that impact meat flavor. In this study we identified volatiles and amino acids from dry and wet aged USDA Prime and Choice strip loins aged for 21 and 42 days that may be associated with off-flavor intensity.

**Materials and Methods:** Forty-eight short loins (IMPS 174; 24 Prime and 24 Choice) were arranged to a 2x2x2 factorial design including aging method (dry and wet), USDA quality grade (Choice and Prime), and aging time (21 and 42 d) as fixed effects. Dry-aged samples were held at 2°C ±2, humidity was maintained at 80-85%, and air speed at 2 m/sec. Wet-aged samples were stored under same temperature in their original vacuum sealed bag. Volatiles were captured by using a SPME fiber in the headspace of a vial containing cooked steak aliquots. Fibers were desorbed on a Shimadzu GCMS-QP2010 SE (Shimadzu Co., Kyoto, Japan) and compounds were identified by retention time, target and qualitative ions and quantified using known standards in five-point calibration curve. Amino acids were also identified and quantified by GCMS using an Agilent 7890A GC System coupled to an Agilent 5975C inert XL MSD with triple-axis mass detector (Agilent Technologies, Santa Clara, CA). Amino acids were identified and quantified using standards provided with the EZfaast Amino Acid kit (Phenomenex<sup>®</sup> Inc., Torrance, CA, US). Correlations presented in this report were obtained by evaluating volatile and amino acid profile and off-flavor intensity data previously reported by the authors during the RMC in 2020 and 2021. Data were analyzed using PROC CORR of SAS.

**Results:** Overall, pentanal was positively and acetoin negatively correlated with off flavor intensity. Pentanal, Alanine, and Proline were positively correlated to off-flavor intensity in Choice- graded strip loins. In USDA Prime loins, Acetoin was negatively correlated with off-flavor intensity and Ornithine negatively correlated. When loins were dry aged, Acetoin, sum of ketones, and sarcosine were negatively correlated to off-flavor intensity. Pentanal, aspartic acid, methionine, and proline were positively correlated with off-flavor intensity when loins were wet-aged. When loins were aged for 21 days, 2,3-Butanedione and sum of ketones were negatively correlated to off-flavor whereas for loins aged for 42 days, pentanal was positively and acetoin was negatively correlated with off-flavor intensity.

**Conclusion:** Pentanal seems to be the most important volatile associated to off-flavor intensity whereas lower levels of acetoin seem to decrease off-flavor perception. Some amino acids including alanine, methionine and proline may also be associated to off-flavor intensity.

Keywords: amino acids, beef, dry-aging, volatiles



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### LIPID CONTENT OF GROUND BEEF INFLUENCES THE RATE OF DISCOLORATION AND LIPID OXIDATION DURING SIMULATED RETAIL DISPLAY

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**Objectives:** Lipid oxidation and myoglobin oxidation result in the development of oxidative rancidity and discoloration in meat, respectively. Previous investigations have documented the potential interrelationship between the two oxidative processes, however, applied research that directly investigates the interactive roles of these processes is limited. Thus, the objective of current study was to examine the relationships between lipid content, lipid oxidation, and discoloration rate of ground beef during a simulated retail display.

**Materials and Methods:** Beef inside rounds (IMPS#168) and bone-in ribs (IMPS#107) from the right-side of steer carcasses (n = 138) were utilized in this study. For each carcass, two grinds of ground beef were manufactured by grinding cubes of inside round and subcutaneous fat from bone-in ribs through a Sirman Master 90 Y12 meat grinder at targeted levels of 10% and 25% fat. A total of two hundred seventy-six 4.54-kg batches of ground beef were manufactured, and six patties were prepared from each batch. Two patties from each batch assigned for evaluation of lipid content by Soxhlet extraction, and initial lipid oxidation with thiobarbituric acid reactive substances assay were immediately vacuum packaged at -30°C. The remaining four patties assigned for evaluation of instrumental color with a Minolta colorimeter, visual discoloration by two trained panelists, and final level of lipid oxidation were overwrapped with PVC film and allotted to a simulated retail display for 7 days under LED lights at 4 °C. Fatty acid profile was analyzed on subcutaneous fat. Pearson correlation coefficients were determined using PROC CORR of SAS, while relationships between meaningful variables were analyzed with simple linear regression using PROC REG of SAS. Correlations were considered weak at |r| < 0.35, moderate at  $0.36 \le |r| < 0.67$ , and strong at  $|r| \ge 0.68$ . Coefficients of determination ( $r^2$ ) were considered weak at  $r^2 < 0.12$ , moderate at  $0.13 \le r^2 < 0.45$ , and strong at  $r^2 \ge 0.46$ .

**Results:** Greater lipid content was strongly correlated with the decrease in  $a^*$  (r = -0.70; P < 0.01), and the increase in  $\Delta E$  (r = 0.68; P < 0.01), whereas lipid content was moderately correlated with the increase in visual discoloration (r = 0.53; P < 0.01). Likewise, lipid oxidation rate was moderately correlated with the decrease in  $a^*$  (r = -0.50; P < 0.01), and the increase in  $\Delta E$  (r = 0.52; P < 0.01), however, lipid oxidation rate was weakly correlated with the increase in visual discoloration (r = 0.26; P < 0.01). A positive moderate correlation (r = 0.36; P < 0.01) was observed between lipid content and lipid oxidation. Fatty acid composition was weakly correlated (|r| < 0.35; P < 0.01) with lipid oxidation and lipid content. Lipid content was a strong predictor for  $\Delta a^*$  (r<sup>2</sup> = 0.49), and  $\Delta E$  (r<sup>2</sup> = 0.47). Lipid oxidation could be moderately ( $0.13 \le r^2 < 0.45$ ) predicted by instrumental color and lipid content, and weakly (r<sup>2</sup> = 0.07) predicted by visual discoloration rate.

**Conclusion:** Lipid content played a more critical role in discoloration compared with lipid oxidation and fatty acid composition. Lipid oxidation could be more reliably predicted by lipid content and instrumental color compared with visual discoloration. Overall, greater lipid content leads to greater rates of lipid oxidation and discoloration.

Keywords: color stability, fatty acid profile, ground beef, lipid content, lipid oxidation



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### NATIVE BEEF COLLAGENASE MAY CONTRIBUTE TO POSTMORTEM COLLAGEN DEGRADATION AND ALTERATION OF CONNECTIVE TISSUE TEXTURE.

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**Objectives:** Collagen is one of the main components in the connective tissue (CT) and contributes to background toughness in beef. It is known that in living animals, collagen can be degraded and remodeled by collagenase matrix metalloproteinases (MMPs); however, it is unclear if collagenase MMPs can impact CT texture during postmortem aging of beef. Therefore, this study aimed to characterize native beef collagenase activity and its relationship to beef tenderness in 3 different cuts and four different aging periods.

**Materials and Methods:** *Longissimus lumborum* (LL), *Gluteus medius* (GM), and *Gastrocnemius* (GN) muscles from both sides were acquired from ten USDA choice beef carcasses. Each muscle was fabricated into steaks and aged at 2 ± 2° C for four different aging periods: 3, 21, 42, and 63 days. Warner-Bratzler Shear Force (WBSF), connective Tissue Shear Force (CTSF), trained panel, collagen content, and collagenase MMPs activity through collagen zymography using bovine type I collagen were performed.

**Results:** As expected, WBSF results indicated LL had the lowest WBSF among all (P < 0.01), with no difference found between GM and GN (P > 0.10). Steaks increased in tenderness from 3 to 21 days of postmortem aging, but no further improvement was found beyond 21 days (P < 0.01). CTSF revealed an interaction between muscle and aging (P < 0.05). LL decreased in CTSF from 3 to 21 to 63 days of postmortem aging (P < 0.05), while LL samples from 42 days were not differ from 21 or 63 days (P > 0.10). GN at 3 days aging had the greatest CTSF value among all but softened quickly (21 days) and remained the same beyond 21 days postmortem aging (P < 0.01). On the other hand, GM showed no difference in CTSF among the aging periods (P > 0.10). Trained panelists reported that GN and GM did not differ in CT content from each other (P > 0.10) and had greater CT amount than LL (P < 0.01). In addition, the trained panelists also noticed more CT amount at 3 days aging steaks than steaks from the rest of the aging periods (P < 0.05). GN and GM did not differ in collagen content (P > 0.10) and had greater collagen content than LL(P < 0.01). However, no aging effect was found for collagen content (P > 0.10). Two distinct sets of unknown collagenase MMPs activity were detected at 72 and 92 kDa in the collagen zymography. The 72 kDa MMP had the greatest activity at 3 days aging, which the activity decreased from 3 to 21 to 42 days (P < 0.01), and no further decrease was found beyond 42 days of postmortem aging. Seventy-two kDa MMP also had the greatest activity in GN muscle compared to the others (P < 0.01). An interaction between aging and muscle (P < 0.05) was found for the 92 kDa MMP, which showed that GN 63 days had greater 92 kDa MMP activity than GN at 3 days postmortem (P < 0.01), while the LL and GM did not differ within the aging periods for the 92 kDa MMP activity (P > 0.10).

**Conclusion:** The findings from this study provided proof that native collagenase MMPs are active in postmortem beef muscles. Both CTSF and trained panels indicated connective tissue softening during postmortem aging, but no change in collagen content was observed. Therefore, we suspect the collagenase MMPs do not work by removing collagen but by altering the collagen structure. Further studies are needed to understand the mechanism of action for the collagenase MMPs in beef.

Keywords: Beef, Collagen, Connective Tissue, Matrix Metalloproteinases, Tenderness



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### A PROPOSED ELISA METHOD TO EVALUATE ELASTIN CONTENT IN BEEF SHANK AND UNDERSTANDING ELASTIN CONTENTS EFFECT ON BEEF SHANK TENDERNESS

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**Objectives:** Collagen and elastin are both major proteins of connective tissue. Collagen and its role in beef tenderness has been extensively studied, but there is no documentation on the effect of elastin on cooked beef tenderness. This is mostly due to the lack of a convenient and reliable methodology to accurately quantify elastin content in meat. Desmosine is an amino acid that is unique to elastin; and perhaps, this amino acid can be used to quantify elastin content in meat. Therefore, the objective of this study was to determine if a competitive enzyme-linked immunosorbent assay (ELISA) method for desmosine can be used to estimate the elastin content in beef and investigate elastin's relationship to cooked beef shank tenderness.

**Materials and Methods:** Six different shank muscles, 3 from the forshank [biceps brachii (BB); deep digital flexor (DDF-F); extensor carpi radialis (ECR)] and 3 from the hindshank [flexor digitorum superficialis (FDS); deep digital flexor (DDF-H); a combination of long digital extensor, medial extensor and peroneus tertius (LMP)] were collected from 8 USDA choice beef carcasses (n = 48). The shank muscles from the right were designated as raw, and the shank muscles from the left were cooked (stewed in water for 90 minutes at 93°C). The samples were hydrolyzed in 6M HCl, and the elastin content was determined for the raw and cooked shanks using ELISA. Briefly, high binding microwell plates were coated with desmosine ovalbumin, incubated and blocked with a blocking buffer. The standards and samples were diluted with the blocking buffer and added to the plate, along with an antidesmosine primary antibody. After incubation, the secondary antibody was added, followed by the addition of tetramethyl benzoate to initiate a reaction between the peroxidase and the substrate. Finally, the reaction was quenched by the addition of sulfuric acid and read at 450 nm using a spectrophotometer. A 4-parameter logistic regression equation was generated using commercially available desmosine standards, and we were able to achieve a coefficient of determination (R<sup>2</sup>) of 0.99. In addition, beef backstrap samples were used as a positive control, and the elastin content of beef backstrap using the proposed competitive ELISA method was ~5.8 mg/ g of muscle tissue, which is comparable to the value provided by Bendall (1967).

**Results:** Of the 6 muscles evaluated, BB, DDF-F, ECR, FDS, DDF-H and LMP had an average elastin content of 2.29, 1.03, 0.68, 0.45, 1.13 and 0.85 mg/g of muscle tissue, respectively. The BB had the greatest level of elastin content compared to the other shank cuts (P < 0.01), and cooked beef shanks had greater elastin content than raw beef shanks (P < 0.05). However, correlation analysis indicated there is no relationship between elastin content and cooked beef shank shear force (P > 0.10).

**Conclusion:** This study demonstrated that elastin content differed among locomotive beef muscles, possibly due to differences in functional needs. In addition, it further confirmed that elastin is heat and water insoluble. Finally, elastin's lack of correlation to cooked beef shank tenderness is possibly due to elastin content being relatively insignificant compared to collagen in connective tissue of muscle. However, this proposed competitive ELISA methodology may be suitable to accurately determine elastin content in beef.

Keywords: beef shanks, connective tissue, elastin, ELISA, tenderness



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### EFFECT OF ROTENONE ON GROUND BEEF COLOR

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**Objectives:** Oxygen consumption (OC) is an inherent muscle property that influences fresh beef color. Furthermore, greater OC results in less bloom and darker meat color, whereas lower OC produces a bright-red color that is more desirable to consumers for purchase and consumption. In post-mortem muscles, mitochondria and oxygen-consuming enzymes are primarily involved in OC and are important in characterizing beef color changes. Although previous studies reported the role of mitochondria in beef color, limited studies have validated mitochondria's role in color. Here we discuss the effect of complex I mitochondrial inhibitor, rotenone, on fresh meat color. Therefore, the objective of this study was to validate the role of mitochondria on meat color.

**Materials and Methods:** Chuck steaks were purchased from a local retail store and were coarse ground. Treatments included a control without rotenone, 0.1, and 0.2% rotenone. All treatments were vacuum packaged and stored for 24 hours to react. The coarse ground beef was then finely ground, hand-formed to 115 g patties, and packaged in polyvinyl chloride film. The patties were kept in a retail display case for three days. Surface color, oxygen consumption (OC), metmyoglobin reducing activity (MRA), lipid oxidation, pH, and NADH content were determined during three days of display. The experiments were replicated three times, and the data were analyzed using the Mixed Proc of SAS. **Results:** There was no effect (P > 0.05) of rotenone addition on pH levels. The addition of rotenone improved (P < 0.05) redness of the patties compared with rotenone. The addition of NADH. Additionally, NADH levels were lower (P < 0.05) in rotenone-treated samples than non-rotenone treated samples. Lipid oxidation was lower in rotenone-treated samples than in control patties. MRA can occur by three different pathways, and in the current research, only complex I was blocked with rotenone.

**Conclusion:** In conclusion, decreased lipid oxidation and other MRA pathways may have increased MRA and redness. Inhibition of all complexes will provide additional insights into the role of mitochondria in beef color.

Keywords: Beef color, Mitochondrial inhibitor, Oxygen consumption, Rotenone



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### DIFFERENCES IN EARLY POSTMORTEM PH AND TENDERNESS VALUES OF BEEF LONGISSIMUS THORACIS MUSCLE ARE INFLUENCED BY METABOLISM AND APOPTOTIC PATHWAYS

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**Objectives:** Understanding how early postmortem (PM) pH and energy metabolism drive differences in beef tenderness is necessary to establish methods to improve and predict beef tenderness. The hypothesis tested was: beef *Longissimus Thoracis* (LT) muscle metabolome and proteome differences at 1 h PM dictate early PM pH linked to variations in early PM beef tenderness.

**Materials and Methods:** Twenty beef steers were slaughtered. Temperature and pH (1, 3, 6, and 24 h) were measured on the LT. Beef LT rib sections were sorted by 6 h pH values to low (LpH; n=9; <5.55 pH; pH range: 5.37-5.54) and high (HpH; n=8; >5.84 pH; pH range: 5.85-5.98) pH classifications. Warner-Bratzler shear force (WBS) was determined on steaks (2.54 cm; 1, 3, 7, or 14 d) cooked to 68°C. LT muscle samples (1 h, 1, 3, 7, and 14 d PM) were frozen. Protein degradation was determined by Western Blotting. Sarcoplasmic protein extracts (1 h PM) were used to conduct 2D-DIGE. Nontargeted metabolomic analyses were conducted on muscle extract (1 h PM) using GC-MS. Temperature, pH, WBS, and protein degradation data were analyzed using the Mixed procedure of SAS v.9.4 (fixed effect=classification; significance of  $P \le 0.05$ , trends of  $0.05 < P \le 0.10$ ). MetaboAnalyst 5.0 (Xia Lab, McGill, CA) was used to analyze metabolites; significance was set at a fold change (FC) of  $\ge 1.25$ ;  $P \le 0.10$ . Melanie 9 (Cytiva, Marlborough, MA) was used to conduct paired t-tests to determine protein abundance differences (FC  $\ge 1.10$ ;  $P \le 0.10$ ).

**Results:** LpH had a lower pH at 1 (P=0.01), 3 (P<0.01), and 6 (P<0.01) h PM, and tended to have a lower 24 h pH (P=0.07), and a greater temperature at 1 (P=0.08) and 24 (P=0.07) h PM. WBS was lower at 1 d PM (P=0.01) and greater at 7 d PM (P=0.05) in LpH. Classification did not affect WBS at 14 d PM (P=0.23). At 1 d PM LpH had greater desmin degradation (P=0.01), a trend for greater troponin-T degradation (P=0.08), and greater calpain-1 autolysis (P=0.01). Proteome analysis at 1 h PM detected a greater abundance of proteins involved in energy metabolism and lesser abundance of proteins involved in apoptosis in LpH (Table 1). Metabolite analysis at 1 h PM revealed greater abundance of succinate (FC= 2.58, P<0.01), lactate (FC= 1.32, P=0.01), glucose (FC= 3.43, P=0.05), and glucose-6-phosphate (FC= 1.37, P=0.07), and less pyruvate (FC= 0.51, P=0.09) in LpH.

Protein	Spot #	FC (LpH/HpH)	<i>P</i> -value (≤0.10)
Phosphoglycerate mutase	10	1.13	0.01
Enolase 3	89	1.14	0.04
Glycogen phosphorylase	154	1.13	0.04
Glycogen phosphorylase	155	1.11	0.09
Creatine kinase M type	169	1.26	0.06
Triosephosphate isomerase	26	1.22	< 0.01
Heat shock 27-kDa protein	28	0.40	< 0.01
Heat shock 27-kDa protein	29	0.67	< 0.01
Malate dehydrogenase	50	1.17	0.03
Malate dehydrogenase	54	1.25	0.09

### Table 1. Proteins differing between 6 h pH classifications at 1 h PM



**Conclusion:** LpH had lower WBS values at 1 d PM due to greater protein degradation. Differences in protein degradation could be explained by calpain-1 autolysis. Energy metabolism proteins and metabolites in glycolytic pathways are linked to PM pH values and could be drivers of LpH phenotype. Similar 14 d WBS values were achieved between pH classifications, however, LpH achieved this sooner through earlier proteolytic mechanisms.

Keywords: beef, metabolome, pH decline, proteome, tenderness



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SURFACE COLOR VARIATION BETWEEN ANGUS AND BRAHMAN LONGISSIMUS LUMBORUM MUSCLE

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**Objectives:** Meat color is a major factor that affects consumer purchasing decisions. Myoglobin (mb), a sarcoplasmic heme protein, is the primary pigment responsible for the color of postmortem muscle. Both mitochondria content and activity may influence color development. Previously, we have shown that Brahman and Angus exhibit variation in postmortem metabolism and mitochondrial function. Thus, the objective of this study was to evaluate color, mb, and mitochondria content in *longissimus lumborum* (LL) from Brahman and Angus.

Materials and Methods: Angus and Brahman steers (n=14 per breed), reared in the same conditions, were harvested at the University of Florida meat lab. Samples were collected from the LL at 1 and 24h postmortem, immediately frozen in liquid nitrogen, and stored at -80° C. At 48h postmortem, carcasses were ribbed, and steaks were removed. The L\*, a\*, and b\* were taken using a Hunterlab spectrophotometer and used to calculate hue and chroma. An additional LL sample was collected at 48h and stored at -80° C. The pH of the LL at 48h was determined using muscle homogenate. Myoglobin concentration (mg/g LL; 1, 24, and 48h) was assessed by absorbance at 525 nm using spectrophotometry. Protein expression of mb, the mitochondrial marker citrate synthase (CS), and complex IV subunit (cytochrome c oxidase subunit IV, COX IV) were evaluated (1 and 24h) using Western blotting. Data were analyzed using an unpaired t-test in SAS-JMP. **Results:** Breed influenced L\* (P < 0.0001) and a\* (P = 0.01) as well as b\* (P < 0.05), with Angus exhibiting higher values. Chroma demonstrated more (P < 0.05) color saturation in Angus compared with Brahman. The pH was not different between breeds (P = 0.89). The mb concentration was numerically higher in Angus at all times; mb tended to be higher at 1h (P = 0.06) and 48h (P = 0.09) but was not different at 24h (P = 0.40). Similarly, protein expression of mb differed between breeds at 1h (P = 0.03) but not 24h (P = 0.44). Breed did not influence protein expression of CS at 1h (P = 0.43) or 24h (P = 0.34). However, COX IV tended to be higher in Brahman at 1h (P = 0.09) and 24h (P = 0.05). Conclusion: Angus displayed greater redness, yellowness, lightness, and color saturation in the LL compared with Brahman. As CS was not different, the mitochondrial content of postmortem LL is likely not related to the increase in surface color values of Angus. The greater mb concentration in Angus evidences greater capacity for oxygen storage, whereas the lower COX IV may contribute to decreased mitochondrial activity. Together, this suggests that altered balance between oxygen storage capacity and mitochondrial oxygen consumption contributed to color differences between breeds.

Keywords: color, mitochondria, myoglobin



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### NOVEL GENES AND MICRORNAS AS BIOMARKERS FOR MEAT TENDERNESS

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**Objectives:** Molecular signatures of beef tenderness indicate that protein biomarkers associated to tenderization/proteolysis pathways may serve as predictors for tenderness. Genes including *CAPN2*, *CAPN15*, *CAST*, *CASP3*, and *PSMC4* and proteins translated from other genes that modulate muscle growth and development have been identified as potential biomarkers for tenderness. In our previous studies, we observed that the expression of microRNAs (miRs) may increase post-mortem. Therefore, expression of certain genes in live animals or at day 0 postmortem may not properly predict tenderness after 14 days of aging. We evaluated the correlation between the expression of 8209 genes with WBSF values of beef aged 0 and 14 days, and the correlation of 315 miRs with WBSF values of beef aged 0, 14, and 28 days.

**Materials and Methods:** Strip loin steaks were aged for 0, 14, and 28 days and 2g aliquots from steaks were obtained and frozen. The remainder of the steaks were cooked and WBSF analysis performed. RNA was isolated from the 2g aliquots via Triazol and quantified and integrity assessed via BioAnalyzer 2100 (Agilent, Santa Clara, CA). Barcoded miRNA-Seq libraries were prepared using the NEXTflex Small RNA Sequencing v3 kit (Bioo Scientific, Austin, Texas) with randomized adapters according to the recommendations of the manufacturer. The fragment size distribution of the libraries was verified via micro-capillary gel electrophoresis on a Bioanalyzer 2100 (Agilent, Santa Clara, CA). The libraries were quantified by fluorometry on a Qubit instrument (LifeTechnologies, Carlsbad, CA), and pooled in equimolar ratios. Twelve libraries were sequenced on one lane of a HiSeq 4000 sequencer (Illumina, San Diego, CA) with single-end 100 bp reads. The large and small RNA sequencing data were summarized by the Salmon and SPORTS computational pipelines, respectively. Transcripts per million (TPM) and reads per million (RPM) were used as the unit for mRNA and miRNA expression, respectively. The differentially expressed genes and miRNAs were identified by the edgeR tool. Level of significance was P<0.05.

**Results:** As expected, tenderization/proteolysis pathway genes CAPN2, CASP3, and PSMC4 significantly decreased for aged beef (14 days; *P*< 0.05). Expression of CAPN15 and CAST was statistically similar when comparing non-aged vs. aged beef (0 versus 14 days). None of those genes were directly correlated to WBSF.

Our data showed that other 42 genes that directly correlated to WBSF values of beef aged 0 and 14 days but only *ACTN4, ERF,* and *MYL12B* modulate enzymes and proteins that have been associated to meat tenderness or cell death. The remaining 39 genes were associated to other metabolic pathways. The expression of 19 miRs was significantly different across 0, 14, and 28 days. Correlations between miRs and WBSF values were observed for 7. Bta-miR-1434, bta-miR-2404-1, bta-miR-2404-2, bta-miR-2427, bta-miR-2478, and bta-miR-2484 were positively correlated to higher WBSF values and the bta-miR-499 was negatively correlated to higher WBSF.

**Conclusion:** Genes associated with metabolic pathways that are not directly related to meat tenderization and proteolysis may better predict meat tenderness when compared to genes such as *CAPN2, CAPN15, CAST* and *CASP3*. The increase or decrease in expression of miRs postmortem indicates that miRs may also be used as biomarkers for tenderization from day 0 to 28.

Keywords: beef, biomarker, microrna, tenderness



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EFFECTS OF OXYGEN ON NON-ENZYMATIC METMYOGLOBIN REDUCTION IN-VITRO

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**Objectives:** Predominant oxymyoglobin imparts consumer-preferred bright cherry-red meat color, but discoloration on meat negatively impacts purchasing decisions. Oxymyoglobin oxidation results in the formation of brown-colored metmyoglobin. Oxidation can occur more readily in common retail settings such as exposure to light and oxygen (O<sub>2</sub>); however, meat has an inherent ability to reduce the brown pigment and form a cherry-red color through metmyoglobin reducing systems. Non-enzymatic metmyoglobin reduction (NMR) indicates metmyoglobin occurs in the absence of enzymes or mitochondria. Research indicated that inherently present electron donors and carriers could contribute to NMR activity. Previous research from our laboratory has demonstrated that the presence of light increased NMR activity in comparison to dark storage. However, limited knowledge is available on the impact of oxygen levels on non-enzymatic reduction. The objective of this study was to evaluate the effect of oxygen levels on NMRA *in-vitro*.

**Materials and Methods:** Solutions of ascorbate and NADH were used as the electron donors and cytochrome *c* (cyt-c) and methylene blue (MB) served as electron carriers. Equine metmyoglobin solution at pH 5.6 was combined with different electron donors and carriers in a 96-well plate. Six treatments included in the study were: 1) NADH; 2) NADH + MB; 3) NADH + MB + ethylenediaminetetraacetic acid (EDTA); 4) NADH + cyt-c + EDTA; 5) ascorbate + MB + EDTA; 6) ascorbate + cyt-c + EDTA. Each treatment was added into a clear bottom 96-well plate with each well containing a total of 300 microliters. A high oxygen gas blend of 80% oxygen and 20% carbon dioxide or a pure 99.9% nitrogen gas was bubbled in phosphate buffers and myoglobin solutions to create two oxygen levels (low-oxygen and high-oxygen). An Ocean Optics Neofox oxygen sensor was utilized to measure oxygen content in solutions. Metmyoglobin reduction was monitored every 5 min for 25 min using a spectrophotometer set to 582 nm. The experiment was replicated three times with six technical replicates per treatment. The data were analyzed using the Mixed Procedure of SAS with a completely randomized design.

**Results:** The oxygen levels in reaction mixtures were 160% and 4%, respectively, in high- and low-oxygen treatments. The percentage levels were relative to the amount of oxygen remaining in the solution following flushing. There was a significant treatment by oxygen effect on the metmyoglobin reduction. An increase in NMR (P < 0.0001) was observed for both combinations of NADH + MB + EDTA and MB + NADH in a high oxygen atmosphere in comparison to a low oxygen atmosphere. The metmyoglobin reduction was limited (P > 0.05) in the presence of NADH alone and within the combination of NADH + cyt-c + EDTA for both oxygen atmospheres. Interestingly when cyt-c was used as an electron carrier, there was no (P > 0.05) oxygen level-specific effect.

**Conclusion:** The current research indicates that level of oxygen has substrate-specific effects on NMR. In conclusion, characterizing the factors affecting metmyoglobin reducing activity helps to develop strategies to improve meat color.

Keywords: Meat color, Metmyoglobin reducing ability, Myoglobin



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### USING PLIMB GENERATED HYDROXYL RADICALS TO DETERMINE SOLVENT ACCESS TO THE HEME POCKET OF HNE ADDUCTED BOVINE MYOGLOBIN

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**Objectives:** The mechanism of aldehyde-induced oxidation of myoglobin resulting in loss of red color requires further characterization. This work utilized Plasma Induced Modification of Biomolecules (PLIMB) to generate hydroxyl radicals and footprint the heme pocket of both 4-hydroxy-2-nonenal (HNE) adducted and non-HNE adducted bovine myoglobin. One hypothesized mechanism of myoglobin oxidation is based on solvent access to the distal heme pocket that can be examined by PLIMB.

**Materials and Methods: Plasma Induced Modification of Biomolecules (PLIMB)**. A ten kilohertz signal of 10 volts was generated and amplified to 10 kV which was then discharged from a steel needle 1mm above protein in buffer. Fifty  $\mu$ L of myoglobin (20  $\mu$ M) in sodium phosphate buffer (50mM, pH 6.5) was used per exposure. Experimental replicates of both the protein type (2) and time of exposure (4) were used for a combined total of 16 samples.

**Orbitrap MS/MS**. After a trypsin/LysC digest, samples were cleaned with a C18 OMIX tip. Samples were injected into a Pepmap C18, 3  $\mu$ M, 100A, 25  $\mu$ M ID, 15 cm reversed phase column and ionized with an EASY-Spray Ion Source. Samples were analyzed with an Orbitrap Elite in data dependent MS/MS mode and with Protein Metrics Byos software. Plasma-induced modifications of side chains included I) mono-oxidation as +15.99 Da, II) di-oxidation as +31.99 Da, III) His to Asp as -22.032 Da, IV) His to Asn as -23.016 Da, V) Lys to Arg as +28.006 Da, VI) Carbamidomethyl as +57.021 Da, VII) Gln to pyro-Gln as -17.026 Da, VIII) Glu to pyro-Glu as -18.010 Da.

**Results:** There are 17 amino acids within four angstroms of the heme iron-protoporphyrin ring that collectively form the distal and proximal pockets, and all 17 residues were examined for oxidative modification from PLIMB. Only five residues (K42, Y103, F138, H64 and I107) were found to be modified, composed of three proximal and two distal pocket residues. With PLIMB exposure of 0, 0.25, 0.25 and 0.75 seconds, only F138 was found to have modification above one percent and show clear dose dependency. Validated modifications of F138 included mono-oxidation (+16 da) and di-oxidation (+32 da).

Statistically, ANOVA (n=16) was used to determine both dose dependency of PLIMB exposure and treatment effect (HNE adducted vs. non-HNE adducted) of the F138 residue. Interestingly, solvent access was trending higher (p< 0.09) in non-adducted myoglobin compared to HNE-adducted myoglobin for the F138 residue. This data suggests that while HNE adducted myoglobin undergoes more rapid oxidation, the mechanism of that oxidation may be outer sphere whereby the distal histidine is not overly protonated by solvent entering the heme pocket. Instead, the heme iron is oxidized by a non-associated oxygen.

**Conclusion:** The study demonstrates the potential for the use of PLIMB in conjunction with tandem mass spectrometry to study solvent access to myoglobin. F138 appears to be more modified in non-HNE adducted myoglobin which suggests that the outer sphere mechanism may be the dominant mechanism of heme oxidation that explains the greater observed oxidation in HNE adducted myoglobin. If the inner-sphere mechanism were responsible for the different rates of oxidation, then the distal histidine (or other residues that form the heme pocket) would be expected to be more modified in the HNE adducted myoglobin, but that effect was not observed.

Keywords: 4-Hydroxy-2-nonenal, Heme, Myoglobin, Oxidation, Protein Footprinting



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## FUNCTIONAL CHARACTERISTICS OF PEROXIREDOXIN-2 UNDER IN VITRO CONDITIONS MIMICKING EARLY POSTMORTEM SKELETAL MUSCLE

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**Objectives:** Protein oxidation adversely affects meat tenderness development. Peroxiredoxins are a family of thiol antioxidant proteins. The form and abundance of peroxiredoxin-2 (Prdx2) vary in pre-rigor and post-rigor porcine skeletal muscle and are associated with meat tenderness and animal phenotype. The cellular events and environments that generate variation in Prdx2 are not defined. Moreover, the impact of Prdx2 on the development of fresh meat quality is not clear. Therefore, the objective was to conduct controlled *in vitro* experiments with partially purified Prdx2 to enhance the understanding of Prdx2 in postmortem skeletal muscle. It was hypothesized that Prdx2 is rapidly oxidized by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and oxidized Prdx2 is less susceptible to degradation by calpain-1.

**Materials and Methods:** Peroxiredoxin-2 preparations from the *diaphragm* (DIA), *psoas major* (PM), and *longissimus lumborum* (LL) were used. Partially purified Prdx2 (35-50% pure) from all muscles was dialyzed against 40 mM Tris-HCl (pH 7.0) and 1 mM EDTA (TE) or TE- 2-(N-morpholino) ethanesulfonic acid (pH 6.0) for 2 h at 4°C. Prdx2 (2  $\mu$ g) was incubated with either 100 or 500  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 2, 15, or 60 min on ice. Prdx2 was incubated with purified porcine calpain-1 in the presence and absence of 2-mercaptoethanol (2ME). Prdx2 (2  $\mu$ g) was adjusted to 1 mM CaCl<sub>2</sub> and incubated with 0.025 U purified calpain-1 for 15, 60, 180, or 1440 min on ice in the presence of 0.5% 2ME. To remove residual 2ME, Prdx2 and calpain-1 were dialyzed against TE pH 7.4. Prdx2 (2  $\mu$ g) was reacted with 500  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 1 h on ice, after which CaCl<sub>2</sub> was adjusted to 1 mM. Purified calpain-1 (0.05 U) was added to each tube and incubated for 15, 60, 180, or 1440 min on ice. After each *in vitro* incubation, denaturing buffer was added to each tube. Prdx2 was detected with immunoblot techniques using monoclonal rabbit anti-peroxiredoxin-2 antibody (ab109367; ABCam, Cambridge, UK). All *in vitro* incubation time was used as a fixed effect, and gel was used as random effect.

**Results:** At pH 7.0, the Prdx2 monomer and dimer decreased after incubation with  $100 \mu$ M H<sub>2</sub>O<sub>2</sub> between 2- to 60-min (*P*<0.05). The Prdx2 dimer increased at pH 7.0 after incubation with  $500 \mu$ M H<sub>2</sub>O<sub>2</sub> between 2- and 60-min (*P*<0.05). At pH 6.0, the Prdx2 monomer decreased, while the dimer increased after incubation with 100 and 500  $\mu$ M H<sub>2</sub>O<sub>2</sub> between 2- and 60-min (*P*<0.05). The intact Prdx2 monomer decreased (*P*<0.05) after 180- and 1440-min incubation with calpain-1 in the presence of 2ME. In the absence of 2ME, the Prdx2 dimer decreased (*P*<0.05) after 180- and 1440-min incubation with calpain-1 in the DIA, decreased after 1440 min in the PM but did not change in the LL.

**Conclusion:** Peroxiredoxin-2 from porcine skeletal muscle reacts with H<sub>2</sub>O<sub>2</sub> at pH 6.0 and 7.0. Calpain-1 was able to degrade Prdx2 monomer and dimer; however, the Prdx2 degradation product that was generated *in vitro* has not been observed previously and may be limited in postmortem meat. The variation in Prdx2 form and abundance from previous meat tenderness studies may be due to Prdx2 solubility or oxidation state differences, such as being present in the hyperoxidized form. Prdx2 may influence the extent of protein oxidation in early postmortem skeletal muscle and promote meat tenderness development.

Keywords: calpain-1, hydrogen peroxide, oxidation, peroxiredoxin-2, porcine



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### A PROPOSED REEVALUATION ON THE IMPACT OF FEEDING DISTILLERS GRAINS PLUS SOLUBLES ON MEAT QUALITY USING A GOAT MEAT MODEL

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**Objectives:** Effect of feeding distillers grains on ruminant meat quality has been well researched in the past decade. However, some studies have suggested that feeding distillers grains can have negative impacts on meat quality, while some demonstrated no negative impact, potentially even improved meat shelf-life in some cases. Corn is inherently rich in antioxidants; as the starch is converted to ethanol, the rest of the grain constituents are concentrated in the distillers grains, including the antioxidants. Therefore, the idea is that feeding distillers grains to livestock will ultimately increase the antioxidant capacity in meat, thus counterbalancing the negative impact from the expected fatty acid alteration in meat. The objective of this study was to evaluate the effect of feeding distillers grains on meat antioxidant capacity and overall quality using a goat model.

**Materials and Methods:** Thirty goat kids (21.7 ± 0.8 kg) were fed either a soybean meal-based diet containing 33% dried distillers grains plus solubles (DDGS; n=15) or 0% DDGS (n=15) for 31 d. There were 6 replications (pens) for each treatment. The goats were harvested at the end of the feeding period, and the loins were collected from all 30 goat carcasses at 1 d postmortem. Loins were fabricated into four 2.54 cm chops and overwrapped for retail display. Goat chops were subjectively evaluated for discoloration and objectively measured using a colorimeter for L\*, a\*, and b\* values on d 0, 4, 7, and 10 under retail display conditions. Samples were collected on those days and analyzed for lipid oxidation, fatty acid profile, and hydrophilic and lipophilic antioxidant concentration via oxygen radical absorbance capacity (ORAC) assay.

**Results:** Overall, visual evaluation of discoloration and meat color characteristics measured by L\*, a\*, and b\* values confirmed that feeding DDGS to goats had no effect on goat chop discoloration (P > 0.10). However, all chops demonstrated a display effect, which they increased in visual discoloration and decreased in a\* values (P < 0.01) over the entirety of the 10-d period of retail display, regardless of the dietary treatment. In addition, lipid oxidation followed the same trend as discoloration, in which the lipid oxidation value was not affected by diet but increased as the display days increased (P < 0.01). As expected, feeding 33% DDGS to goats decreased relative % of multiple monounsaturated fatty acids (MUFA), primarily on fatty acids C16:1, C17:1 and C18:1 and total MUFA content (P < 0.05). At the same time, dietary intake of 33% DDGS increased relative % of multiple polyunsaturated fatty acids (PUFA), primarily on fatty acids C18:2, C20:5 and C22:5, and total PUFA (P < 0.05). The ORAC displayed no treatment difference in the hydrophilic antioxidant activity (P > 0.10), but chops from the 33% DDGS dietary treatment had greater lipophilic antioxidant activity compared to the 0% DDGS chops (P < 0.05).

**Conclusion:** Results from this study illustrated that feeding DDGS to goats will alter fatty acid composition in goat meat, but the supposed negative consequence from increased PUFA is likely counterbalanced by the increased antioxidant capacity in the lipid component of meat, resulting in no difference in meat shelf-life. Ultimately, this study explained the cause of the variability in meat shelf-life from ruminants supplemented with various forms of distillers grains.

Keywords: discoloration, distillers grains, goat meat, lipid oxidation, shelf life



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### EFFECTS OF SUCROSE AND BEEF SUPPLEMENTATION DURING MID TO LATE GESTATION ON FETAL MUSCLE GROWTH AND DEVELOPMENT USING A SOW BIOMEDICAL MODEL

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**Objectives:** Myogenesis in pigs is a biphasic process where the primary generation of fibers (from 35 to 55 gestation day) are surrounded by secondary fibers (from 55 to 95 gestation day) creating a rosette shape, and finally an external ring of third generation fibers (late fetal to early postnatal period). Primary fiber number is believed to be a fixed genetic effect, while secondary fibers are influenced by prenatal events such as maternal diet. **Objective:** The aim of this study was to compare the effects of beef or sugar supplemented to a standard sow maternal diet on fetal muscle fiber development. **Materials and Methods:** 21 pregnant sows (Landrace × Yorkshire, starting BW = 222 ± 35 kg) were randomly assigned to 1 of 4 isocaloric supplement treatments: control (CON) 126g corn-soybean meal-based diet (CSM), 110g cooked ground beef (BEEF), 85.5g sucrose (SUG), or the combination of 54.8g BEEF and 42.7g SUG (BS). Dietary supplements were added three times per day to the CSM from day 40 to 110 (±0.58) of gestation. Sows were euthanized on d 111 of gestation; *longissimus dorsi* (LD) and *semimembranosus* (SM) samples were collected from 1 median weight male and female fetus of each sow. Samples were then immunofluorescent stained to target the slow and fast twitch muscle fiber types and analyzed through fluorescent microscopy to obtain the total amount of myofibers and the proportion of slow (ST) and fast twitch (FT) fibers. Data were analyzed using the MIXED procedure of SAS with sow diet, fetal muscle, and fetal gender as fixed effects, and replicates as a random effect with individual sow as the experimental unit. Differences were considered statistically significant at P < 0.05.

Treatment*	Fetal muscle⁺	Slow twitch m	nuscle fibers	Fast twitch muscle fibers	
		Estimate (%) <sup>1</sup>	standard error	Estimate (%) <sup>1</sup>	standard error
CON	LD	6.81 <sup>bc</sup>	0.93	93.19 <sup>bc</sup>	0.93
	SM	4.78 <sup>ab</sup>	0.93	95.22 <sup>ab</sup>	0.93
BS	LD	5.98 <sup>ab</sup>	0.96	94.02 <sup>ab</sup>	0.96
	SM	4.57 <sup>ab</sup>	0.96	95.43 <sup>ab</sup>	0.96
SUG	LD	9.19 <sup>c</sup>	0.88	90.81 <sup>c</sup>	0.88
	SM	5.30 <sup>ab</sup>	0.88	94.70 <sup>ab</sup>	0.88
BEEF	LD	6.79 <sup>bc</sup>	0.85	93.21 <sup>bc</sup>	0.85
	SM	4.22ª	0.85	95.78ª	0.85

Percentage of slow and fast twitch myofibers in *longissimus dorsi* and *semimembranosus* of swine fetal muscles at 111 d of gestation.

\*Control (CON) = 126g corn-soybean meal-based diet (CSM); BEEF = 110g cooked ground beef; SUG = 85.5g sucrose; BS = 54.8g BEEF + 42.7g SUG.

\*Longissimus dorsi (LD) and semimembranosus (SM).

<sup>1</sup>Values with different letter indicate significance at P<0.05.

**Results:** Muscle type (LD vs. SM) differed for total amount (P < 0.0001) and myofiber percentage (P < 0.0001). LD showed the largest percentage of ST (SUG > BS, but did not differ from CON and BEEF ) and greatest total number of myofibers (CON had the highest, 5562/1mm<sup>2</sup>); in contrast, SM showed the largest percentage of FT (BEEF = 95.8%), and the smallest value of total amount (SUG = 3372/1mm<sup>2</sup>). It was found that female LD and SM had a numerically greater percentage of ST and smaller myofiber number than male but did not significantly differ.



**Conclusion:** These results suggest that this amount of beef or sucrose supplementation to a standard sow maternal diet had minimal effects on fetal muscle fiber type.

Keywords: beef, diet, myofiber, sugar, swine model



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## EFFECTS OF WET AGING ON DIFFERENTIAL PROTEIN ABUNDANCE IN DARK-CUTTING AND NORMAL-PH BEEF LONGISSIMUS LUMBORUM MUSCLES

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**Objectives:** Dark-cutting beef is a meat quality defect associated with a very dark-meat surface color when the cut surface is exposed to oxygen. As a result, dark-cutting beef is discounted at retail due to its appearance. The greater than normal muscle pH can be associated with dark-cutting condition. However, the effects of high pH meat on regulatory mechanisms mediating protease activity or in destabilizing methyl-transferases of protein complexes during the aging process in dark-cutting compared with normal-pH beef is still unknown. The objective of the current research was to determine the differential protein expression levels with aging in dark-cutting and normal-pH beef longissimus muscles.

**Materials and Methods:** *Longissimus lumborum* (LL) muscles from 12 different animals (6 dark-cutters and 6 USDA Choice strip loins) were collected within 48 h post-mortem. Loins were sectioned into equal sections and randomly assigned to 7 and 14 days of aging, respectively. Following aging, steaks were cut, placed on foam trays, wrapped with polyvinyl packaging film, and allowed to bloom for 1 h. Steaks from Choice and dark-cutting loins were utilized to measure pH, surface color, and proteomic analysis.  $L^*$ ,  $a^*$ ,  $b^*$ , and chroma were recorded using a HunterLab MiniScan spectrophotometer. Muscle samples were also subjected to quantitative proteomics analysis using LC-MS/MS-based proteomics. Surface color data were analyzed using the Mixed Procedure of SAS. Proteomics data were analyzed using several bioinformatics approaches, and the aging-related changes in protein expression profiles were considered significant at a false discovery rate (FDR) < 0.05.

**Results:** Aging time increased (P < 0.05)  $a^*$ ,  $L^*$ , and chroma values for dark-cutting beef, while only numerical increase (P > 0.05) was observed in USDA Choice steaks. Mass spectrometry analysis identified 1,000 proteins, of which 283 showed significant differential abundance of aging-related changes (FDR < 0.05) between dark-cutting and USDA Choice beef. Quantitative analysis revealed that aging mainly affected protein abundance in high pH meat (dark-cutting) compared with Choice beef. Of the 282 differentially expressed proteins, 95 were up-regulated, and 25 were down-regulated on day 7 postmortem in dark-cutting beef. In contrast, 25 proteins were up-regulated and 49 were down-regulated on day 14 (fold change > 1.5, FDR < 0.05) in dark-cutting beef. Majority of the up-regulated proteins in dark-cutting beef on day 7 were involved in redox homeostasis, chaperon and protease mediated stress responses. Conversely, cytosolic proteins involved with energy metabolism processes such as glycolysis, glycogen degradation, glycerol-phosphate shuttle, stress, and heat shock proteins were down-regulated by day 14 postmortem in dark-cutting beef.

**Conclusion:** Overall, our finding reveals different aging-related proteolytic degradation mechanisms in dark-cutting compared with normal-pH beef. Understanding the biochemical basis of dark-cutting beef has implications for increasing economic benefits associated with effective and targeted enhancement interventions for improving the color of dark-cutting beef.

Keywords: Aging, Dark-cutting beef, Mass spectrometry, Meat color, Proteomics



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### DIFFERENCES IN THE METABOLOME OF BEEF LONGISSIMUS THORACIS MUSCLE FROM STEERS SUPPLEMENTED SUPRANUTRITIONAL ZINC AND RACTOPAMINE HYDROCHLORIDE COULD EXPLAIN EARLY POSTMORTEM PH DECLINE AND TENDERNESS VARIATIONS

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**Objectives:** Ractopamine hydrochloride (RAC) and supranutritional zinc (Zn) influence pH values and tenderness development. The hypothesis was that differences in the early postmortem (PM) metabolome and proteome of *Longissimus Thoracis* (LT) muscle from beef cattle supplemented supranutritional Zn and RAC influence pH values and early PM tenderness.

Materials and Methods: Angus crossbred steers were assigned to dry rolled corn diets based on growth potential and initial body weight: non-Zn supplemented control (CON-NO; 36 mg Zn/kg dry matter; n=5), supranutritional Zn supplementation (SUPZN-NO; CON diet+ 60 ppm Zn from ZnSO₄+ 60 ppm Zn from Zn amino acid complex; n=5), CON+ RAC supplementation (CON-RAC; 300 mg RAC-steer<sup>1</sup> d<sup>-1</sup>; n=5), and SUPZN+ RAC supplementation (SUPZN-RAC; n=5). Zn treatments were fed for the entire 89 d trial. RAC was fed for the final 28 d for RAC treatments. On 5 different harvest dates, 1 steer per treatment was harvested (n=4/d). LT pH values were taken (1, 3, 6, and 24 h PM). LT muscle samples were taken at 1 h and 1 d PM, and frozen. Warner-Bratzler shear force (WBS) values were determined on cooked (68°C) steaks (2.54 cm; 1, 3, 7, or 14 d PM). Western blot analysis was used to determine protein degradation (1 d PM). Muscle extracts (1 d) were used for nontargeted metabolomics using GC-MS. Sarcoplasmic protein extracts (1 d PM) were used for LC-MS/MS with tandem mass tagging. WBS, pH, and protein degradation data were analyzed as a 2x2 factorial using the mixed procedure of SAS v.9.4 (fixed effects= Zn, RAC, and the interaction; block= harvest date). Significance was  $P \le 0.05$  and trends of  $0.05 < P \le 0.10$ . Metabolomic data were analyzed using MetaboAnalyst 5.0. Significance was a fold change (FC) of  $\geq$ 1.25; P<0.10. Proteomic data were analyzed using t-tests. Significance was set at a P<0.10. **Results:** Interactions were not significant; main effects will be presented. At 6 h PM, Zn supplementation tended to lower (P=0.06) LT pH compared with non-Zn supplemented steers. LT from RAC fed steers had a greater pH (P=0.04) 6 h PM than non-RAC supplemented steers. RAC supplementation resulted in a greater (P<0.01) WBS and Zn supplementation tended for a lower (P=0.06) WBS at 1 d PM compared with non-RAC and Zn supplemented samples, respectively. RAC supplementation resulted in less desmin (P=0.05) and troponin-T (P=0.04) degradation at 1 d PM compared with non-RAC supplemented samples. Differences in glycolytic, fatty acid and other energy metabolites were identified (Table 1). SUPZN-NO had less AMP deaminase and greater malate dehydrogenase than CON-NO or SUPZN-RAC. SUPZN-NO had greater pyruvate dehydrogenase subunit beta than CON-NO or CON-RAC. CON-RAC had more fructose bisphosphate aldolase and glyceraldehyde phosphate dehydrogenase than CON-NO or SUPZN-RAC. Image:



Metabolite	Comparison	FC <sup>1</sup>	P-value 0.05 0.08 0.08
Glucose	CON-RAC/CON-NO	1.67 1.43 1.38	
Glucose-6-Phosphate	CON-RAC/CON-NO		
Glucose	CON-RAC/SUPZN-NO		
Glucose-6-Phosphate	CON-RAC/SUPZN-NO	1.45	0.05
Linoleic Acid	CON-RAC/SUPZN-NO	0.57	0.10
Linoleic Acid	CON-RAC/SUPZN-RAC	0.58 0.56 0.59	0.08 0.01 0.09
Oleic Acid	SUPZN-RAC/CON-NO		
Oleic Acid	SUPZN-NO/CON-NO		
Glucose	SUPZN-NO/CON-NO	2.00	0.06
Lactic Acid	SUPZN-NO/CON-RAC	1.41	0.05
Sorbitol	SUPZN-NO/CON-NO	2.50	0.02
Sorbitol	SUPZN-NO/CON-RAC	4.17	0.01
Sorbitol	SUPZN-NO/SUPZN-RAC	2.10	0.03

#### Table 1. Metabolites differing in abundance between samples supplemented RAC and supranutritional Zn at 1 d PM

<sup>1</sup>FC values are expressed as treatment 1/treatment 2. A FC of greater than 1.00 is greater in abundance in treatment 1. A FC of lesser than 1.00 is lesser in abundance in treatment 1.

**Conclusion:** Differences in glycolytic metabolites and enzymes represent explanations for variations in LT pH early PM. Fatty acid metabolites could represent differences in use of the beta oxidation pathway for energy production in CON-RAC and SUPZN-NO. Greater sorbitol in SUPZN-NO represents shifts to the polyol pathway in PM energy production influencing a lower pH at 6 h PM and lower WBS values at 1 d PM. These metabolic profile differences demonstrate the influence of nutritional management on meat quality development.

Keywords: beef, metabolism, metabolome, pH decline, proteome



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MITOCHONDRIA MORPHOLOGY IN POSTMORTEM LONGISSIMUS LUMBORUM OF ANGUS AND BRAHMAN

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**Objectives:** Mitochondria (Mt) have the potential to contribute to early postmortem metabolism which could affect proteolysis and meat quality. Previously, we showed that Mt function in *longissimus lumborum* (LL) declines during the first 24h postmortem, and Brahman Mt sustain function longer than Angus. The objective of this study was to assess ultrastructural morphology of Mt in LL postmortem using transmission electron microscopy.

**Materials and Methods:** Angus and Brahman steers (n=14 per breed) were reared in similar conditions and harvested at approximately 19 months of age. Samples of LL were collected at 1, 3, 6, and 24h postmortem, sliced with a razor blade, and cut into 1 mm<sup>3</sup> cubes. Samples were treated with a fixing solution, dehydrated with ethanol and placed in LR-White resin to cure. The samples were placed into grids and stained sequentially with 1% sodium metaperiodate, 2% uranyl acetate, and 3% lead citrate. A subset of samples (Angus, n=4; Brahman, n=5; 1 and 24h) were then examined under the transmission electron microscope and scanned in at least three different sections per grid. Images of intermyofibrillar and subsarcolemmal Mt were taken. Only Mt with a defined outer membrane were evaluated and quantified using Fiji-ImageJ. Mitochondria were analyzed for circularity, roundness, surface area ( $\mu$ m<sup>2</sup>), perimeter ( $\mu$ m), optical density (0=white, 1=black; describes matrix density), Feret's diameter ( $\mu$ m; longest distance between two points), and aspect ratio. Data were analyzed in JMP Pro 15.0 using a mixed model with fixed effects of breed (b), time (t), and their interaction (b×t). Time was considered a repeated measure.

**Results:** Circularity, roundness, surface area, perimeter, optical density, Feret's diameter, and aspect ratio were not affected by breed (b, P > 0.1) or the interaction (b×t, P > 0.1). However, time influenced surface area (P < 0.05), perimeter (P < 0.05), and Feret's diameter (P < 0.05) with greater values at 24h. Optical density tended to differ by time (P = 0.07), with lower values at 24h.

**Conclusion:** Breeds exhibited similar trends in Mt shape and matrix density. However, the increased surface area, perimeter, and Feret's diameter from 1 to 24h postmortem evidence Mt swelling. The lower optical density at 24h is also consistent with Mt swelling, indicating increased permeability of inner and outer membranes. Analysis of Mt at 3 and 6h is needed to establish progression of morphological changes and their relationship with Mt functional parameters and proteolysis between breeds.

Keywords: mitochondria, transmission electron microscopy



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### NUTRIGENOMICS: AVAILABILITY OF BEEF-DERIVED MICRORNAS AFTER DIGESTION MAY MODULATE GENE EXPRESSION AFTER MEAT CONSUMPTION

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**Objectives:** MicroRNAs (miRs) are small single-stranded non-coding RNAs with an average of 22 nucleotides in length, which are known to silence genes and/or inhibit protein translation. These RNAs are unique, as miRs are resistant to aging and cooking, possibly due to the protection against denaturation offered by the miR-induced silencing complex (miRISC) comprised by Argonaute and other proteins. In this study we evaluated the effects of cooking and *in vitro* digestion on the availability of beef-derived miRs to determine if they may be available at the duodenum for further absorption.

**Materials and Methods:** *Longissimus lumborum* steaks were removed from four (n=4) cross-bred steers 15 min *post-mortem*, ged for 14 d and cooked and digested. Steaks were cooked until temperature reached 158°F and an aliquot of approximately 2 grams was digested with pepsin and trypsin to mimic human digestion. RNA was extracted and isolated from samples via Triazol. All samples were quantified and measured for RNA integrity via BioAnlyzer 2100 (Agilent, Santa Clara, CA). Barcoded miRNA-Seq libraries were prepared using the NEXTflex Small RNA Sequencing v3 kit (Bioo Scientific, Austin, Texas) with randomized adapters according to the recommendations of the manufacturer. The fragment size distribution of the libraries was verified via micro-capillary gel electrophoresis on a Bioanalyzer 2100 (Agilent, Santa Clara, CA). The libraries were quantified by fluorometry on a Qubit instrument (LifeTechnologies, Carlsbad, CA), and pooled in equimolar ratios. Sixteen libraries were sequenced on one lane of a HiSeq 4000 sequencer (Illumina, San Diego, CA) with single-end 100 bp reads. Data were summarized by the SPORTS computational pipelines. Counts per million (CPM) were used as the unit for expression. Differentially expressed miRNAs were identified by the edgeR tool. **Results:** 

As expected, here was a decreased expression for many MiRs, but these non-coding RNAs did not disappear completely suggesting resistance to cooking and digestion. Unexpectedly, several miRs had significantly increased expression in digested beef compared to fresh beef (P<.05). A total of 413 miRBase registered bos taurus miRs were detected in digested samples. MicroRNAs with highest logCPM counts in digested samples were: bta-miR-1-2 (26.97%), bta-miR-1-1 (26.97%), bta-miR-451 (8.66%), bta-miR-486 (6.97%), bta-miR-143 (1.78%), bta-miR-92a-1 (1.77%), bta-miR-133a-2 (1.69%), bta-miR-133a-1 (1.68%), bta-miR-92a-2 (1.54%), and bta-miR-22 (1.37%). Higher expression levels of bta-miRs 1434, 760, 1484, 193-b, 345, 677, 1248-1, 1248-2, 1296, 1307, 320a-1, and 320a-2 were observed in digested when compared to fresh beef.

**Conclusion:** Predictions between 90-100 suggest that human homologous miRs hsa-miR-1-3p and hsa-miR-1-5p may modulate the expression of 190 genes associated to several metabolic pathways. Therefore bta-miR-1-2 and bta-miR-1-1 may also modualte human genes. Target scores above 80 suggest that modulations are likely to be real. This supports the hypothesis that miRs play an important role in nutritional values of meats if they are absorbed in the intestines. In vivo studies are currently underway to confirm that miRs can be absorbed, reach the blood stream, and modulate the expression of inflammatory (e.g. IL-6) and metabolic genes (e.g. PEPCK) in the gut and associated tissues such as the liver.

Keywords: beef, microrna, nutrigenomics



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### EXPLORING THE POTENTIAL EFFECT OF ANTI-PHOSPHOLIPASE A2 ANTIBODY TO EXTEND BEEF SHELF-LIFE IN A BEEF LIPOSOME MODEL SYSTEM

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**Objectives:** Phospholipase-A<sub>2</sub> (PLA2) is a ubiquitous enzyme that cleaves a fatty acid tail at the sn-2 position from a phosphatidylcholine (PC), an abundant phospholipid (PL) class in cell membranes. The resulting free fatty acids (FFA) are typically polyunsaturated fatty acids (PUFA) which are prone to lipid oxidation when exposed to pro-oxidants such as light and oxygen. A PLA2 antibody (aPLA2) can be mass produced through laying hens, and the egg powder containing aPLA2 has been used as a feed supplement to improve growth performance for various livestock. We hypothesize that the aPLA2 from the egg powder may inhibit PLA2 activity, preventing the formation of FFA, which can potentially improve the shelf stability of beef. Therefore, the objective of this study was to utilize a beef liposome model system to investigate this proposed mechanism.

**Materials and Methods:** Total lipid was extracted from 10 USDA choice beef loins at 3d post-mortem. The PL was separated from each lipid pool through solid phase extraction. The PL from each steak was further split into six different treatments: 1) PL (10mg/ml of PL); 2) aPLA10 (PL+10ug/ml of aPLA2); 3) aPLA20 (PL+20ug/ml of aPLA2); 4) PLA2 (PL+4ug/ml of PLA2); 5) PLA2+aPLA10 (PL+PLA2+10ug/ml of aPLA2); 6) PLA2+aPLA20 (PL+PLA2+20ug/ml of aPLA2). Treatments were mixed with a tris/CaCl<sub>2</sub> buffer and incubated at 37°C for 2 hrs to activate PLA2. After the incubation, an aliquot was immediately taken for PL profile analysis by mass spec. Eighty  $\mu$ M of bovine myoglobin was added to the remaining samples and exposed to retail display conditions (4°C; 2300 lx) for 7d. At 0, 1, 4, and 7d, aliquots were taken for lipid oxidation analysis.

**Results:** There was a display x treatment interaction for lipid oxidation (P < 0.01). At 7d display PLA2, PLA2+aPLA10, and PLA2+aPLA20 treatments had greater lipid oxidation compared to the samples without PLA2. Interestingly, not only did 7d aPLA10 and aPLA20 have less lipid oxidation than PL only and all PLA2 treatments, but 7d aPLA10 and aPLA20 also had less oxidation compared to 4d PLA2 (P < 0.01). This trend continued to be seen in the other retail display periods. As expected, PL profile analysis showed clear differences between treatments with or without PLA2. The PLA2 treatments showed greater relative % of total lysophosphatidylcholine (LPC) and LPC 16:0, 16:1, 18:0, and 18:1 than treatments without PLA2 (P < 0.01). The PLA2 treatments had significantly less relative % of total ether-linked PC (ePC) than treatments without PLA2, specifically, ePC34:1, 34:2, 34:4, 36:1, 36:3 and 36:4 (P < 0.05). Interestingly, the PLA2 treatments did not seem to have significant effect on relative % of total PC as seen in ePC. Finally, it appears that aPLA2 had no effect on inhibiting PLA2 hydrolysis as there was no difference between PLA2 and aPLA+PLA2 treatments in relative % of total ePC, PC as well in LPC composition (P > 0.10).

**Conclusion:** This study confirmed that the hydrolysis of PL by PLA2 influenced lipid oxidation. Although no inhibition effect was observed for PLA2 by aPLA2, there appears to be an antioxidant effect for aPLA2 for lipid oxidation. On the other hand, PLA2 appears to attack ePC more effectively than PC. Perhaps, a product ion analysis to reveal the fatty acid composition for each PL species can help explain the reasoning behind why ePC was hydrolyzed more than the traditional ester bonded PC.

Keywords: Beef, Lipid Oxidation, Lipidomic, Phospholipase A2, Phospholipid



## **Technical Summaries**

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## UTILIZING A VIRTUAL INDUSTRY WORKSHOP TO ASSESS THE INSTRUCTIONAL EFFECTIVENESS OF A MEAT PROCESSING COURSE

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**Objectives:** The objective of this project was to use a virtual industry workshop to assess the instructional effectiveness of a meat processing course via student participation and student survey feedback.

Materials and Methods: Beyond Fresh Meats (BFM, Tyson) was used to assess the instructional effectiveness of an undergraduate/graduate cross listed (Animal Science and Food Science) meat processing course (ANSC/FSTC 447/667 Industrial Processed Meat Operations (IPMO)). Students (N=7) had been exposed to functional properties of proteins/lipids, ingredient technology, comminuted, restructured and whole muscle products, marination/injection, thermal processing, batter and breader processing and product development prior to BFM. BFM objectives were to utilize current ingredients, processes, and equipment to gain a deep understanding of marination and batter/breading in poultry and red meat products and to provide students an industry perspective and exposure to what a career in R&D entails. BFM was conducted in five lecture/lab modules with support packages (seasonings, raw materials, finished products, etc.). BFM modules were: Module 1- Processing Lecture/Virtual Plant Tour; Module 2 - Functional Ingredients & Poultry Raw Materials; Module 3 – Flavors, Seasonings, Marination; Module 4 – Batter and Breading and Module 5 – Cross Functional Team Engagement. Students participated in BFM lectures at the designated times and BFM lab activities were conducted during two IPMO lab times (Lab 1-Modules 1/2; Lab 2 Modules 3/4/5). Surveys were emailed to students after the conclusion of BFM. Students were asked to respond to guestions on a 1-5 scale: 1-Strongly disagree and 5-Strongly agree. The survey posed questions for each BFM module and on how well IPMO prepared them for each BFM module and if information/activities should be included in IPMO. Surveys were anonymous. **Results:** For Module 1, students indicated that virtual tours should be a part of ANSC 467/667 (4.43). Students specified that IPMO lecture and lab information helped them understand the information provided in BFM Module 2 - 5.00 and 4.86 and Module 3- 4.71 and 4.86 (lecture and lab, respectively). Students stated that BFM Module 2 information on Poultry Raw Materials should be included in IPMO (4.14). Slightly lower values were observed for BFM Module 4 where students' average rating was 4.57 (lecture) and 4.00 (lab). Students rated IPMO as a 4.71 (lecture) and 5.00 (lab) for Module 5 which included information on sensory protocols, regulatory and nutritional requirements, and collaborationsales/marketing for developing new products. This was possibly due to the IPMO team R&D projects the students were engaged in. Survey data showed that participation in the virtual BFM course enhanced student understanding of processed meat operations (4.86). Students indicated that BFM enhanced their understanding of information/concepts presented in IPMO (4.57) and the use of virtual demonstrations should be included in IPMO (4.43). Conclusion: BFM enhanced students' knowledge and understanding of processed meat manufacturing. Course improvements to IPMO should focus on providing more information/labs on poultry raw materials and batter and breaded products. The research and development team project should remain as the class capstone event. Development of virtual demonstrations (used as a pre-lab activity) would enhance student learning.

Keywords: assessment, Beyond Fresh Meats, instruction, meat processing