

Graduate Student Research Poster Competition

Ph.D. DIVISION

Antimicrobial Resistance of *Salmonella* spp. Isolates Found on Beef Animal Hides and Carcasses, and the Potential Ramifications for Producers

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In assessing human health ramifications of administering antimicrobial drugs to livestock, quantifying antimicrobial resistance levels for enteric pathogens and commensal microorganisms is imperative. According to FDA-CVM's "Framework Document" for evaluating and minimizing human health effects associated with antimicrobial use, safety considerations for antimicrobials must include their importance in human medicine to establish resistance thresholds. This study evaluated resistance of *Salmonella* isolates, from beef hides and carcasses in eight packing plants, to tetracycline, streptomycin, sulfonamides, ampicillin, and chloramphenicol. In each plant, hide and carcass sponge swab samples were obtained before dehiding and before carcass chilling. Of 639 samples, 49 hide and 4 carcass samples yielded 521 confirmed *Salmonella* spp. isolates. Of 53 confirmed samples, 17 (32.1%), 17 (32.1%), 11 (20.8%), 8 (15.1%) and 8 (15.1%) yielded 104, 44, 36, 22 and 22, respectively, isolates resistant to tetracycline, streptomycin, sulfonamide, ampicillin and chloramphenicol, respectively. Ampicillin and chloramphenicol resistance was, individually, restricted to eight samples yielding 22 penta-resistant isolates. All 67 isolates obtained in one plant were solely resistant to tetracycline, accounting for 91.8% of isolates matching that profile. Except for samples (one each) yielding isolates simultaneously resistant to tetracycline and streptomycin or tetracycline and sulfonamides, resistant isolates were penta-resistant or resistant only to a single antimicrobial. If tetracycline, streptomycin, sulfonamides, and ampicillin are classified as Category II drugs in FDA-CVM's "Framework Document", resistance frequency, coupled with human exposure risk associated with their presence on carcasses, illustrates importance of regulating antimicrobial use and reiterates need for producers to follow Judicious Antimicrobial Use Guidelines.

Technique Differences to Isolate *E. coli* O157:H7 from Beef Hides

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This study compared suggested USDA methods and an Immunomagnetic Separation (IMS) procedure for sensitivity in detecting *E. coli* O157:H7 on beef hide samples when time before enrichment and sample handling procedures varied. A 2 method \times 3 time/handling factorial was used. Three sterile sponges were used to aseptically sample a 7.6 \times 38 cm area of beef hide, then halved and placed in sterile sampling bags. Thirty animals were sampled over 5 d. Samples were randomly assigned to a treatment group of Method 1 - Immediate (M1-I), Delayed Chilled (M1-DC), or Delayed Room Temperature (M1-DRT), or the same handling practices under Method 2 (M2-I, M2-DC, M2-DRT). Method 1 used USDA procedures utilizing the BioControl Assurance EIA EHEC test kit for screening where Method 2 utilized Dynal anti-O157 magnetic beads. Immediate samples began enrichment within 4 h of sampling. Delayed samples were placed in ice chests with icepacks (DC) or without icepacks (DRT), and held at room temperature 28 h. Method 2 increased the likelihood of finding samples that were confirmed by O agglutination when compared to Method 1 (77.8 vs 8.9%; $P < .05$). Immediate testing found 26 O agglutination positive samples compared to 22 positive samples by both DC and DRT. All O positive samples from Method 1 were non-identifiable with the api 20 E test. However, of the 70 positive samples after O agglutination in Method 2, 25 (35.7%) were identified as *E. coli*. Within Method 2, immediate enrichment identified 66.7% of samples, DC 36.7%, and DRT 30.0%.

Spectrophotometric Determination of Lamb Tissue Peroxide Value

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Flavor is the predominant characteristic of meat responsible for consumer preferences. One of the most important causes of flavor deterioration in meat is lipid oxidation that begins almost instantly after slaughter. Lipid oxidation in fresh lamb tissue (n = 6) and a corn oil control (n = 6) were measured using two peroxide value assays: 1) AOCS peroxide method and 2) a new spectrophotometric peroxide method (SPM) in addition to the 2-thiobarbituric acid (TBA) method. PVs determined by the AOCS and SPM methods were 1.4 and 1.5 meq/kg ($P \geq 0.05$), respectively. Coefficients of variation (CVs) for AOCS and SPM methods were 33.7% and 10.7%, respectively. The TBA mean of fresh lamb tissue was 0.10 $\mu\text{g/g}$ with a CV of 14.5%. The PVs of corn oil, determined by the AOCS and SPM methods, were 52.2 and 51.7 meq/kg ($P < 0.05$), respectively. The CVs of AOCS and SPM methods were 0.36% and 0.21%, respectively. The TBA mean was 0.39 $\mu\text{g/g}$ with a CV of 4.6%. Low CVs in SPM indicated that it has better reproducibility than either the TBA or AOCS method in measuring lipid oxidation in both lamb tissue and corn oil. In addition, SPM yields results in less than 30 min. These results demonstrate that SPM is more rapid and reproducible than either AOCS or TBA methods and could be an effective method to evaluate lipid oxidation in animal tissue.

Supplementation with Creatine Monohydrate Improved the Lean Quality of Fresh Pork of Two Different Genotypes

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This project examined the effect that 1) supplementing hogs with dietary creatine monohydrate, and 2) the presence of the recessive halothane gene had on the lean quality of various muscles. Supplemented hogs gained more ($P < 0.05$) weight over 5 d of supplementation. There also were trends toward increased objective marbling scores and lower cooking loss ($P < 0.10$). The 45-min pH was higher ($P < 0.05$) for supplemented hogs in the semimembranosus (SM); however, there were no treatment effects on objective color or muscle composition. Genotype had an effect on most quality measures, with carcasses from carrier hogs producing lean that subjectively and objectively had poorer quality. The recessive halothane gene caused less desirable subjective color and marbling scores ($P < 0.05$), and a trend ($P < 0.10$) toward higher shear force values. In addition, halothane carrier carcasses had lower 45-min pH of the longissimus thoracis (LT), a higher drip loss for the LT and biceps femoris (BF), and a lower L^* value for the SM and BF (all $P < 0.05$), with a trend

toward lower L^* for the LT and semitendinosus (ST) ($P < 0.10$). Genotype also affected the composition of several muscles, with the normal genotype having more fat and less moisture. The most important result of treatment was a reduction in the percentage of carcasses that were classified as PSE and RSE. Supplementation reduced the percentage PSE of the LT from 75% to 30%, and the ST from 68% to 38%. These results indicate supplementation with CMH may improve lean quality.

Calcium Modulation Using Vitamin D₃ to Improve Beef Tenderness

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The calcium-activated protease μ -calpain has been implicated as a major causative agent in postmortem tenderization via protein degradation. The calcium dependency of calpains allows for the supplementation of vitamin D₃ to increase Ca⁺⁺ content of muscle enabling activation of calpains and accelerating postmortem proteolysis. Steers (n = 142) were arranged in a 4 × 3 factorial consisting of 4 levels of vitamin D₃ treatment (0, .5, 1, and 5 million IU/steer/d) for 8 consecutive days antemortem consisting of 3 distinct breed-types. Feedlot performance and WBS were measured at 3, 7, 10, 14 and 21 days postmortem for longissimus lumborum, semimembranosus, and supraspinatus steaks. Vitamin D₃ treatment (5 million IU) reduced average daily gain and feed intake (dry matter basis) for the last two days of feeding ($P < .05$). All vitamin D₃ treatments decreased ($P < .05$) longissimus lumborum WBS at 7, 10, 14 and 21 days postmortem and semimembranosus WBS at 3, 7, and 14 days postmortem. Vitamin D₃ treatments decreased μ -calpain activity and increased vitamin D₃ concentrations ($P < .05$) in kidney, longissimus, and plasma. Liver vitamin D₃ concentrations were increased ($P < .05$) by vitamin D₃ treatments of 1 and 5 million IU. These results indicate the supplementation of steers with .5 million IU/steer/d of vitamin D₃ for 8 consecutive days improves WBS in longissimus lumborum and semimembranosus steaks by affecting calcium and μ -calpain, while having minimal effects on feedlot performance and tissue residues.

Transglutaminase Cross-Linking of Whey/Soy/Myofibrillar Proteins and the Effect on Protein Gelation

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Four experiments were conducted to examine the effects of processing conditions on Transglutaminase (TGase)-catalyzed WPI(SPI)-MPI cross-linking (pH 6.0): 1) ionic strength

(0-0.6 M NaCl), 2) enzyme:substrate ratio (0.1% TGase:2-8 mg/mL protein), 3) buffer/calcium, and 4) preheating of WPI and SPI (80°C, 5-60 min). TGase treatment of MPI/WPI(SPI) in water produced a major band immediately below the myosin heavy chain, accompanied by a concomitant attenuation in the native myosin band, apparently due to forming intramolecular linkages. Also noticeable was the disappearance of actin. These electrophoretic changes reached a maximum after 5 min and 1 h, and then were reversed after 2 h and 4 h, for SPI and WPI, respectively, indicating a reversible nature of the cross-linking. Addition of NaCl gradually diminished protein changes, and at 0.6 M NaCl, minimum intramolecular interactions were observed while some newly formed poly-

mers (> 200 kDa) were evident. Neither the enzyme:substrate ratio, preheating, nor calcium (5 mM) influenced the enzyme reaction. Reduced intensity in β -conglycinin and glycinin bands suggests that both soy protein components may have interacted with MPI. The enzyme treatment did not change the thermal curves for the WPI/MPI; however, the treatment markedly enhanced the gelling ability (elasticity or G') of the mixed proteins, e.g., the final G' (at 80°C) increased to 400-500 Pa from the control (223 Pa) ($P < 0.05$). Transglutaminase is capable of modifying intra- and intermolecular interactions and rheological properties of muscle proteins; however, the presence of nonmuscle proteins and low ionic strength are essential for the enzyme actions.