months to monitor P excretion and blood urea N. Individual farm advisory teams were formed to provide targeted advice, and were comprised of the owner, nutritionist, veterinarian, extension agent and other key individuals. Each farm advisory team met monthly and reviewed milk yield, milk composition, MUN content, and results of feed analyses to determine if changes were needed to reduce excess excretion of N and P. Advisory teams were used to improve communication, owner education, and implementation of needed changes, but the owner made all final decisions. Nutrient budgets were developed for each farm for N and P at the start of the study and following ration and management changes. Ten control herds similar to collaborator herds were identified and monitored. Feed samples and intake data were collected and analyzed every three months in control herds, and milk yield, milk composition, MUN, and reproductive data recorded once per year. Control herds received this data, but no additional intervention occurred and no advisory teams were established. The goal of this project was to demonstrate the use of nutritional and management practices to reduce nutrient losses from Virginia dairy farms.

Key Words: Farm Advisory Teams, Dairy, Nutrient Losses

450 Nitrogen cycling on pasture based dairies. T.W. Downing*, *Oregon State University, Corvallis*.

Most animal waste management plans (AWMP) written for pastured based dairies use estimates for manure produced and yields removed to design the waste plan. Landowners theoretically have been required to apply nitrogen (N) in quantities equal to what they remove annually in a crop. As concerns for water quality have increased, so has the need to demonstrate that the nutrients applied are equal to what is removed. Over the past year, a trail was conducted on three farms to develop a realistic plan for dairymen to document nutrient application and removal on pasture based dairies. This challenge was fairly complex, because grazing animals are constantly harvesting forage and depositing manure. You also have continuous grass growth as a factor. Participates received a customized AWMP, calibration of manure handling equipment, and a detailed farm map. They also decided how they were going to measure standing forage daily and record their data. Soils samples were taken before and at the conclusion of the project to help verify and confirm the results. Farm A did not complete the yearly forage measurements. Farm B had grass yields ranging from 8743 to 20177 kg/ha with an average of 16926±1959kg. Farm C had yearly yield totals ranging from 4214 to 16254 kg/ha with an average of 8967±3138kg. Pasture protein levels varied some throughout the season, but were averaged to determine the approximate level on nitrogen removed. Total nitrogen removed per hectare by grazing ranged from 225 up to 589kg of N/ha removed. Net nutrient balance for nitrogen was negative for thirty-one of thirty-two fields studied. Farm A continues to record manure applications by field, focusing efforts on even manure distribution. Farm B and C found this increased level of management rewarding and profitable. They also used this data to give them confidence to add commercial fertilizer. Both these farms believe this approach has made them more profitable in addition to being able to truly document agronomic applications.

Key Words: Waste manangement, Nutrient balance, Animal Waste Management Plan

FOOD SAFETY

451 A comparison of antibiotic resistance patterns from swine farms using or excluding antibiotics. M. Beckmann*, F. R. Jackson, and A. G. Mathew, *The University of Tennessee, Knoxville, TN*.

The effects of farm use or exclusion of antibiotics on antibiotic resistance patterns of bacteria were compared using fecal samples from live swine. Four farms that used antibiotics and three farms that excluded antibiotics from production were selected and from each farm, 6 pigs from each of 4 weight groups (4.5, 23, 45, and 109 kg) and 5 sows were randomly selected for collection of fecal samples. Non-pathogenic *E. coli*, O157:H7 *E. coli*, and *Salmonella spp.* were isolated from fecal samples and tested for sensitivity to gentamicin, sulfamethazine, oxytetracycline, ceftiofur sodium, and ampicillin using a standardized minimum inhibitory concentration (MIC) analysis. Sensitivity patterns were markedly different between farm types in non-pathogenic *E. coli*, and moderately so in salmonella. In both cases, isolates from farms that excluded antibiotics had lower ($P < .05$) MICs. The number of resistant isolates and those that demonstrated multiple resistance patterns was greater ($P < .05$) on farms that used antibiotics. Nonpathogenic *E. coli* from farms that excluded antibiotics had significantly lower ($P < .001$) MICs for gentamicin, sulfamethazine, oxytetracycline, and ampicillin and lower ($P < .10$) MICs for ceftiofur. Farm type differences were most evident for isolates from younger pigs for gentamicin, ceftiofur, and ampicillin, but were also noted among all pig groups for sulfamethazine and oxytetracycline. In salmonella, the MICs were higher from farms that used antibiotics, particularly for oxytetracycline and ceftiofur ($P < .001$). O157:H7 *E. coli* were isolated from 2 farms, both of which used antibiotics in production, thus a relevant analysis on that bacterium was not possible. In total, these data indicate that exclusion of antibiotics in swine production decreases antibiotic resistance in non-pathogenic *E. coli*, and to a lesser extent resistance in salmonellae.

Key Words: Antibiotic resistance, *E. coli*, Swine

452 Effect of drug combinations and regimens on antibiotic resistance in bacteria from swine. F. R. Jackson*, M. Beckmann, and A. G. Mathew, *The University of Tennessee, Knoxville*.

In 2 replicate trails, 144 weaned pigs were used to test the effects of antibiotic dosing schemes on resistance in bacteria. Pigs were inoculated with the foodborne pathogen *Salmonella typhimurium* prior to being treated with feed- and water-based antibiotics. Treatments included maximum label use, rotation of similar and non-similar antibiotics, increasing gradient doses, and pulse dosing of antibiotics for a period of 2 weeks following pathogen challenge. Fecal samples were obtained prior to initiation of treatments, on various days during treatment, and throughout the grow-finish phase. The challenge organism and non-pathogenic *E. coli* were recovered from fecal samples and tested against all antibiotics used in the study to determine effects on resistance patterns. Antibiotic resistance was affected to a greater extent in non-pathogenic *E. coli* compared to *Salmonella typhimurium*. Greater resistance ($P < .0001$) occurred when similar antibiotics (apramycin, gentamicin, neomycin) were used in rotation compared to the other treatments. Significant ($P < .05$) time by treatment interactions also occurred during or just following rotational treatment with similar antibiotics compared to samples collected later and from other treatment groups. Pigs on the control and pulse dose treatments produced bacteria with lower resistance compared to other groups. These data indicate that dosing regimens affect antibiotic resistance patterns in bacteria associated with swine.

Key Words: Swine, Salmonella, Antibiotic Resistance

453 Prevalance of verotoxin-producing *Escherichia coli* in sheep grazing Great Basin irrigated pastures. S. L. Lake*, B. H. Thran, H. S. Hussein, S. F. Khaiboullina, M. R. Hall, and H. A. Glimp, *University of Nevada, Reno*.

Although sheep have never been implicated in an *Escherichia coli* associated foodborne illness, the limited research published in recent years have shown that sheep harbor verotoxin- producing *E. coli* (VTEC), including O157:H7 at high rates. This suggests that mutton, lamb, or their products share a food safety risk similar to that of beef. In most cases research has focused on characteristics (i.e. sorbitol negative and 4-methylumbelliferyl-β-D-glucuronide [MUG] negative) usually associated with *E. coli* O157:H7. However, VTEC encompass numerous serotypes of *E. coli* and are not limited to sorbitol negative; MUG negative isolates. The objective of this study was to assess the VTEC prevalence in sheep grazing an irrigated pasture over 6 months (summer and fall, 1999). Twenty yearling (15-mo old) ewes (7/8 Merino; 1/8 Rambouillet) were selected at random from a large flock (>1,000 ewes) at Rafter 7 Ranch. The ewes grazed fescue (*Festula arundinacea*) and white clover (*Trifolium repens*) pasture during the summer and were

supplemented with alfalfa (*Medicago sativa*) hay during winter. Thirty-nine fecal samples were rectally collected in August and November. One ewe was lost to predation after the first collection. Initial *E. coli* isolates were selected using microbiological methods utilizing the lack of sorbitol fermentation properties of *E. coli* in conjunction with MUG. Verocytotoxicity tests were performed to determine the toxicity status of the isolates. Eleven isolates from five ewes (one from the summer and four from the fall collections) were cytotoxic. None of the isolates matched the classical identification of O157:H7 (sorbitol negative; MUG negative) and two isolates were sorbitol negative but MUG positive. All isolates were initially identified as sorbitol negative, but when tested by the API identification system, nine isolates were sorbitol positive. Eight isolates were MUG positive and one isolate was MUG negative. The prevalence rate of VTEC in these ewes was 5% for the summer collection and 20% for the fall collection suggesting that the infection was transient. Based on the characteristics of our isolates, *E. coli* O157:H7 was not the prevalent VTEC in grazing Nevada range land. These results also support the importance of screening sheep for VTEC instead of limiting the tests to O157:H7. Our observations agree with recent studies indicating that *E. coli* O157:H7 is not the predominant VTEC in sheep.

Key Words: Food safety, Sheep, *Escherichia coli*

454 Determining incidence levels of Salmonella and Escherichia coli 0157:H7 on beef cattle. A.R. Barham*[1], G.H. Locke[2], D.M. Allen[2], J.R. Blanton[1], and M.F. Miller[1], [1]*Texas Tech University,* [2]*Excel Corporation*.

The objective of this study was to determine the effects of a pre-slaughter warm water wash, feedlot management practices and season (summer vs, fall) on incidence levels of *Salmonella* (SM) and *Esherichia coli* 0157:H7 (EC) in beef cattle. Immediately following arrival at packing facilities, treated animals received a warm water hide wash. Bacterial hide swipes were aseptically collected following exsanguination and placed in Tryptic Soy Broth (TSB) and shipped to a USDA approved microbiological laboratory (Silliker Laboratory) for microbiological assays. A second group of animals were randomly selected from 12 different feedlots from the West Texas Panhandle and were examined for bacterial contamination upon arrival at the packing facility. Feedlot variables examined were capacity and antibiotic treatments. Feedlots used in the study fed a commercially available grain based diet. Bacterial hide swipes were aseptically collected during the fall (November and December; S1) and summer (June and July; S2) to evaluate seasonal effects. Samples were returned to Texas Tech University's Meat Science Laboratory within 4 hours of sampling and analyzed utilizing USDA approved methods. Statistical analysis of data was performed with SAS CATMOD procedures. No significant differences were obtained with warm water hide washes. Significant differences were detected for SM incidence levels between feedlots ($p<.05$) but was not detected for EC. No significant differences were detected for SM or EC due to feedlot capacity or antibiotic treatment. *Salmonella* incidences were also significantly higher in S2 than S1 ($p<.05$) but no differences were detected for *E. coli*. In conclusion, feedlot and season had a large effect on SM, however antibiotic treatment and feedlot capacity did not influence these incidence levels. No effects were seen on the incidence of *E. coli* for season or feedlot management practices

Key Words: food safety, Salmonella, E. coli

455 *Escherichia coli* O157:H7 becomes resistant to sodium chlorate in pure, but not mixed culture or in vivo. T. R. Callaway*[1], R. C. Anderson[1], S. A. Buckley[1], M. A. Carroll[2], L. F. Kubena[1], and D. J. Nisbet[1], [1]*USDA/Agricultural Research Service-Southern Plains Agricultural Research Center College Station, TX,* [2]*Texas A&M University, College Station*.

Sodium chlorate kills *Escherichia coli* O157:H7 via non-specific reduction of chlorate by nitrate reductase and has been proposed as an additive to ruminant diets prior to slaughter. Cells lacking nitrate reductase are chlorate-resistant, and are easily selected in pure culture. The objective of this study was to determine if chlorate-resistant *E. coli* O157:H7 strains could be selected in vivo. *E. coli* O157:H7 strains (n=13) were screened and all were chlorate-sensitive ($P<0.05$) as determined by specific growth rate; but overnight chlorate-treated cultures (10 mM) became chlorate-resistant. When *E. coli* O157:H7 was incubated in sterilized fecal juice with 10 mM chlorate, CFU/ml declined from 10^6 to 10^3 in 6 h ($P<0.05$), but returned to 10^5 by 24 h, and these cells were chlorate-resistant. When *E. coli* O157:H7 was incubated in fresh fecal fluid with 10 mM chlorate, numbers declined from 10^6 to 10^1 CFU/ml in 6 h ($P<0.05$) and to <10 CFU/ml by 24 h ($P<0.05$) but these colonies were chlorate-sensitive. Continuous culture of *E. coli* O157:H7 (D=0.05) contained 10^7 CFU/ml that were always chlorate-sensitive ($P<0.05$). Addition of 10 mM chlorate to the chemostat caused a transient CFU decrease, but remaining colonies were chlorate-resistant upon testing (n=20). Continuous culture of *E. coli* O157:H7 in fresh fecal juice had a total anaerobic population of 10^{10} cells/ml and *E. coli* O157:H7 of 10^6 CFU/ml. When 10 mM chlorate was added, the *E. coli* CFU/ml declined to 10^3 ($P<0.05$) but no colonies were chlorate-resistant (n=20). When piglets (n=30) were challenged with *E. coli* O157:H7 and treated with 100 or 200 mM chlorate, no chlorate-resistant isolates were cultured from the ileum, cecum, colon or rectum. These results indicate that chlorate-resistant O157:H7 strains can be selected in pure, but not mixed culture and suggests that terminal chlorate feeding will not select for chlorate-resistance in vivo.

Key Words: E. coli O157:H7, Chlorate, Chlorate resistance

456 Tasco Supplementation in Feedlot Cattle: Effects on Pathogen Loads. L.L. Behrends, J.R. Blanton, Jr., M.F. Miller, K.R. Pond, and V.G. Allen, *Texas Tech University, Lubbock*.

The objective of this study was to determine the effects of Tasco-EX (extract from *Ascophyllum nodosum*) supplementation on pre- and post-harvest shedding of *Escherichia coli (E. coli)*, *Escherichia coli* O157 (*E. coli* O157) and *Salmonella* in feedlot cattle. Forty-eight Angus feedlot steers had 0%, 1% and 2% Tasco-EX added to their conventional grain based diets on a dry matter basis 2-wks prior to slaughter. Fecal samples and hide swipes were collected from each animal prior to shipment to the slaughter facility and immediately following exsanguination at the slaughter facility. Samples were returned to Texas Tech University Meat Science Laboratory within 4 hr. of collection. Samples were analyzed for *Salmonella*, *E. coli* and *E. coli* O157 using FSIS and USDA approved protocols. Data were analyzed by ANOVA as a randomized block design and were further tested by orthogonal contrasts to determine linear, quadratic and cubic relationship of treatments. Data revealed that hide and fecal *Salmonella* levels were not significantly affected by Tasco-EX supplementation. However, *E. coli* in fecal and hide samples decreased linearly ($P < .10$) as the percentage of Tasco-EX in the diet increased. More importantly, we found that there were lower ($P < .05$) levels of *E. coli* O157 in treated animals for both fecal samples and hide swipes. In conclusion, Tasco-EX supplementation may be able to decrease *E. coli* incidence levels in feedlot cattle when administered 2-wks prior to slaughter.

Key Words: *E. coli*, *Salmonella*, Food Safety

457 Technique Differences to Enumerate and Isolate *E. coli* O157:H7 from Beef Hides. M. A. Carr*[2], L. D. Thompson[1], C. B. Ramsey[1], M. San Francisco[1], S. P. Jackson[1], and M. F. Miller[1], [1]*Texas Tech University, Lubbock,* [2]*Angelo State University, San Angelo, TX*.

The objectives of this study were to compare the currently suggested USDA method and a procedure including Immunomagnetic Separation (IMS) for sensitivity in detecting *E. coli* O157:H7 on beef hide samples when time before enrichment and sample handling procedures were varied. A 2 method X 3 time/handling factorial was used to examine detection methods and pre-analysis sample handling practices. Three sterile sponges were used to aseptically sample a 7.6 cm X 38 cm area of beef hide and then halved and placed in sterile sampling bags. A total of 30 animals were sampled on 5 days. Samples were randomly assigned to a treatment group of Method 1 - Immediate (M1-I), Method 1 - Delayed Chilled (M1-DC), Method 1 - Delayed Room Temperature (M1-DRT), or the same handling practices under Method 2 (M2-I, M2-DC, M2-DRT). 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 1 used current USDA procedures utilizing the BioControl Assurance EIA EHEC test kit for screening where Method 2 utilized Dynal anti-O157 magnetic beads. The Method 2 increase 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%. By incorporating the IMS techniques with the Dynal anti-O157, the likelihood of confirming *E.coli* O157 was increased.

Key Words: Food Safety, *E. coli* O157:H7, Immunomagnetic Separation

458 Development of Beef Quality Assurance (BQA) programs for cow-calf producers: A glimpse at what worked for North Dakota. G.P. Lardy*, C.S. Stoltenow, and L. Lee, *North Dakota State University, Fargo*.

Most states have implemented a beef quality assurance (BQA) program. The beef industry in North Dakota (ND) consists largely of cow-calf producers and backgrounding operations. In 1998, development of a BQA program, which includes education and marketing components, began in ND. North Dakota's BQA program is funded jointly by the NDSU Extension Service, ND Beef Commission, ND Agricultural Products Utilization Commission, and USDA/FSIS. Other agencies involved in development of the program include the ND Stockmen's Association, ND Livestock Marketing Association, ND Veterinary Medical Association, ND State Department of Agriculture, and allied industry groups, including pharmaceutical companies that are active in the state. At least one representative of each group serves on the state BQA committee. The committee also includes ranchers, seedstock operators, feeders, veterinarians, and a sale barn owner. In 1998 and 1999, the committee spent extensive time securing funding, planning, and setting goals. Materials from several existing state programs were studied prior to the implementation. In early 1999, a program coordinator was hired and the ND BQA manual and corresponding presentation material were developed. Over 35 producer certification meetings were held across the state in the fall of 1999. Over 900 beef cattle producers were certified at these meetings, representing approximately 7.5% of ND beef cattle producers. Our most successful meetings (based on attendance) were held with the help of local veterinarians or sale barn operators. This indicates the importance of local support in determining program success. Our BQA program has been approved by the Nebraska Corn-Fed Beef Program, giving producers access to an additional market outlet and a potential premium. We believe that there are several factors responsible for the program's success including adequate funding, a producer friendly manual, involvement of local sale barns, veterinary clinics, and associated businesses in sponsoring the programs, and a team of people excited about BQA.

Key Words: Beef, Quality, Assurance

459 Reduction of fecal shedding of Enterohemorragic *Escherichia coli* O157:H7 in lambs by feeding microbial feed supplement. M. Lema*, L. Williams, and D. Rao, *Alabama A&M University, Normal*.

Ruminants are reservoirs of *E. coli* O157:H7 and fecal shedding of the pathogen forms the vehicle of entry into the human food chain. We studied the efficacy of *L.acidophilus*, *S. feacium*, a mixture of *L. acidophilus* and *S. feacium* and a mixture of *L. acidophilus*, *S. feacium*, *L. casei*, *L. fermentum* and *L. plantarum* in reducing fecal shedding of *E. coli* O157:H7 by sheep experimentally infected with the pathogen prior to administration with the microbials. Following oral inoculation with 10^{10} CFU of *E.coli* O157:H7, five groups of six Suffolk ram lambs were fed daily for seven weeks a basal diet without microbial supplement (control) or the basal diet with the different pure and mixed microbial supplements. Fecal sample was collected weekly and analyzed for *E. coli* O157:H7 using modified tryptic soy broth with novobiocin as a preenrichement broth and cefixim-tellurite sorbitol MacConkey agar (CT-SMAC) as a selective medium. *E. coli* O157:H7 was confirmed by its reaction with O157 and H7 antisera. Lambs administered a mixture of *L. acidophilus*, *S. feacium*, *L. casei*, *L. fermentum* and *L. plantarum* shed significantly lower ($P < 0.05$) total number of *E. coli* O157:H7 than the other lamb groups over the entire experimental period. *S. feacium* supplemented lambs were comparable ($P > 0.05$) to lambs fed a mixture of *L. acidophulus* and *S. feacium* in fecal shedding of the pathogen but significantly lower ($P < 0.05$) than the control lambs and those supplemented with *L. acidophilus*. Average daily gain (ADG) and gain to feed ratio (G:F) were significantly improved ($P < 0.05$) by the mixed culture microbials.

Key Words: Enterohemorrhagic *E. coli* O157:H7, Fecal shedding, Microbial feed supplement

460 Epidemiological survey of Salmonella prevalence in Pennsylvania dairy herds. H. Aceto*, R.J. Munson, D.T. Galligan, C.E. Benson, D. Munro, and S. Rankin, *University of Pennsylvania School of Veterinary Medicine, Kennett Square*.

This ongoing survey aims to assess the herd prevalence of salmonella (S), specifically *S. typhimurium* DT 104, in PA dairies. A demographic and management survey was designed to identify risk factors for the introduction and maintenance of S infection. Microbiological samples comprise 3 separate milk filters and an environmental sample from the sick/fresh cow area. Isolation of S was by means of standard enrichment and differential plating procedures. To date, surveys are complete on 51 farms. Of those 51, 11 were positive for various S, 4 of which were found to be *S. typhimurium*. Phage-typing identified two of the *typhimuriums* as DT104, one DT208, and one "untypable". One of the DT104 isolates had the pentaresistance (ampicillin, chloramphenicol, streptomycin, sulfonamide, tetracycline) profile that often typifies DT104, and long-PCR revealed the presence of a 10 kb multiresistance gene cluster. The other DT104 was only resistant to ampicillin. Interestingly, both DT104's came from dual-enterprise producers. The DT208 isolate exhibited significant resistance (ampicillin, tetracycline, kanamycin, spectinomycin, streptomycin). The remaining untypable *typhimurium* was fully sensitive. Other serotypes of S isolated were *agona* (1), *thompson* (2), *montevideo* (1), *senftenberg* (1), *derby* (1), *anatum* (2) and *infantis* (1). The majority of which were fully sensitive, or resistant to only one antimicrobial. In most cases (10 of 11 positives), S was cultured from the milk filter. On 4 of those 10 farms, S was found on both the milk filter and in the environmental sample. Four farms were associated with more than one type of S. Collection of milk filters and an environmental sample from the sick/fresh cow area appears to be an effective means of monitoring S on dairy farms. However, S are associated with intermittent shedding, as evidenced by subsequent problems on farms initially found negative, and point-sampling is likely to seriously underestimate the true herd prevalence of S.

Key Words: Salmonella, Prevalence, Milk filter

461 Detection of verotoxin-producing *Escherichia coli* in culled beef cows. H. S. Hussein*, B. H. Thran, M. R. Hall, S. F. Khaiboullina, W. G. Kvasnicka, and R. C. Torell, *University of Nevada, Reno*.

In 1982, the clinical importance of verotoxin-producing *Escherichia coli* (VTEC) was recognized when *E. coli* O157:H7 was isolated from human stools during an outbreak of foodborne illness associated with the consumption of improperly cooked ground beef. Because slaughtered cull cows contribute significantly to the ground beef supply they are considered a food safety risk factor if they harbor VTEC. The objective of this study was to assess the prevalence of VTEC in culled beef cows (Angus, Herford, or their crossbreds) at the time of shipping to slaughter (between the months of September 1999 and January 2000). Fecal samples were rectally collected from 82 culled cows (8 to 12 year-old) representing eight Nevada ranches with average herd size of 600 beef cows, of which 10 to 20% are culled annually. The cows grazed meadow regrowth of range land forages (i.e., crested wheatgrass [*Agropyron desertorum*], bromegrass [*Bromus inermis*], and tall fescue [*Festuca arundinacea*]). Initial isolates were selected using classical microbiological methods based on sorbitol fermentation and 4-methylumbelliferyl-β-D-glucuronide (MUG) properties. Toxicity of the isolates was determined by performing verocytotoxicity tests. Cytotoxic isolates were detected in fecal samples from eight cows (one cow in each of two ranches; three cows in each of two ranches). Eighteen cytotoxic isolates were further characterized with the API identification system to clarify sorbitol fermentation. Five cytotoxic isolates matched the classical identification of O157:H7 (sorbitol negative; MUG negative) and four isolates were sorbitol negative but MUG positive. The remaining nine isolates were confirmed as sorbitol positive, one isolate was MUG negative, and eight were MUG positive. The VTEC prevalence rate ranged from 0 (4 ranches) to 30% (1 ranch). These results demonstrate that the number of VTEC-positive cows differ within a herd and the cytotoxic isolates are of different characteristics. This suggests the importance of screening beef

cattle for VTEC instead of limiting assays to O157:H7. By expanding the detection methods, chances of detecting *E. coli* are increased and possible on-farm management practices that minimize the risk of beef contamination can be implemented. These practices should improve the safety of beef entering the food chain.

Key Words: Food safety, Cull cows, *Escherichia coli*

462 A one-year investigation of prevalence of verotoxin-producing *Escherichia coli* in beef heifers grazing an irrigated pasture. B. H. Thran*, H. S. Hussein, S. F. Khaiboullina, and M. R. Hall, *University of Nevada, Reno*.

Verotoxin-producing *Escherichia coli* (VTEC) are a major cause of foodborne illnesses. In many cases, cattle were implicated as a reservoir of VTEC. Most investigations in the US focused on specific characteristics (i.e. sorbitol negative and 4-methylumbelliferyl-β-D-glucuronide [MUG] negative) associated with O157:H7. The objective of this study was to assess the prevalence of VTEC in beef cattle grazing an irrigated pasture over a one-year period. A herd of 23 yearling Angus heifers grazed grass pasture with only supplementation of alfalfa hay *(Medicago sativa)* during winter. A total of 86 fecal samples were rectally collected during four periods (spring [April], summer [July], fall [October], and winter [December] of 1999). Three heifers were excluded after the second collection for reproduction-related reasons. Using classical microbiological methods utilizing the lack of sorbitol fermenting properties of *E. coli* along with MUG, isolates were selected. Toxicity of these isolates was determined by verocytotoxicity tests. Positive cytotoxicity was detected for nine isolates from five heifers (one in the spring, summer, and fall and two in the winter collections). Four isolates matched the classical identification of O157:H7 (sorbitol negative; MUG negative). Two isolates were sorbitol negative but MUG positive. The remaining three isolates were initially thought to be sorbitol negative but were sorbitol positive (as well as MUG positive) by API identification. The VTEC prevalence rate in our herd ranged from 4.3% (summer or spring) to 10% (winter). Results indicate that VTEC-positive heifers within a herd differ in VTEC characteristics and that the infection is transient. Only one heifer tested VTEC positive in two periods (spring and summer). Recent studies indicated that *E. coli* O157:H7 was a proportion (8.2 to 40%) of VTEC isolated from cattle feces. Therefore, it is important to test beef cattle for VTEC instead of limiting the assays to O157:H7. Identification of VTEC-positive beef cattle before slaughter is a critical step in on-farm strategies to minimize the risk of beef contamination with foodborne pathogens.

Key Words: Food safety, Beef cattle, *Escherichia coli*

463 Stereochemical determination of clenbuterol residues in hogs. D. J. Smith*, *USDA ARS Biosciences Research Lab, Fargo, ND*.

Clenbuterol HCl is a β-agonist bronchodilating agent that has been used in an off-label manner to enhance the leanness of cattle and swine. Consumption of clenbuterol residues has resulted in the intoxication of humans. Cardiovascular side-effects of clenbuterol are attributed to its R stereoisomer. The objective of this study was to determine the stereochemical composition of clenbuterol residues in edible tissue of swine. Nine barrows and nine gilts were provided 1 ppm dietary [^{14}C]clenbuterol HCl for 7-d and were slaughtered (n = 3 per sex) with 3.7 $\pm$ .7, 74.3 $\pm$ .8, and 169.6 $\pm$ .8 hour withdrawal periods, respectively representing nominal 0-, 3-, and 7-d withdrawal periods. Lung was included as an edible tissue based on published reports of clenbuterol intoxication after the consumption of pork-lung soup (World Food Chem News, 1998, 5:13). Clenbuterol was extracted, derivatized with phosgene, and quantified with GC-MS using a validated procedure (Wilson et al., 1994; JAOAC Int. 77:917). Clenbuterol D9 was used as the internal standard. Stereochemical separation was achieved with a Chiraldex^{TM} B-DM capillary column. Elution order of clenbuterol derivatives was determined using HPLC-purified stereoisomers; stereochemical assignment was based on polarimetric measurements. Clenbuterol oxazolidone isomers were detected in the selective ion mode (m/z 243, 245, 302, 304; IS 249, 251, 311, 313) and peak areas of individual ions were summed. Stereochemical compositions were calculated from total peak areas. The stereochemical assay was validated for sensitivity and selectivity. Livers, kidneys, and lungs of 0-withdrawal hogs (n =6) contained greater amounts of the S isomer (68.2 $\pm$ 2.2, 57.3 $\pm$ 1.1, and 56.3 $\pm$ .9%, respectively) than the R isomer (31.8 $\pm$ 2.2, 42.7 $\pm$ 1.1, and 43.7% respectively). The stereochemical composition of clenbuterol-D9 remained constant in incurred and spiked tissue samples (48.9 $\pm$ .2% R and 51.1 $\pm$ .1%). In conclusion, a method was developed which allows the stereochemical determination of clenbuterol stereoisomer residues in tissues of treated animals. The method was used to demonstrate that clenbuterol isomers are retained in a stereoselective manner in edible tissues of hogs.

Key Words: Clenbuterol, Residues, Swine

464 A carbonate and alkali treatment that eliminates *Escherichia coli* from dairy cattle manure. F. Diez-Gonzalez[1], G.N. Jarvis[1], D.A. Adamovich[1], and J.B. Russell*[2], [1]*Cornell University, Ithaca, NY,* [2]*ARS/USDA, Ithaca, NY*.

Escherichia coli persists in manure for long periods of time, and it is a potential source of water and food contamination. Most strains are harmless, but some cattle harbor pathogenic strains (e.g. O157:H7) without showing signs of infection. Viable *E. coli* counts in fresh manure (n = 25) and in farm storage tanks (n = 5) ranged from 100,000 to 100,000,000 per g. When fresh cattle feces were mixed equal parts (1 to 1) with urine, the *E. coli* counts declined, and after 10 days, the viable *E. coli* count was less than 10 cells per g manure. If feces and urine were mixed in a 2.2 to 1, a ratio typical of dairy cattle manure, the *E. coli* count did not decrease. The antibacterial activity of urine could not be explained by urea, alkaline pH, or ammonia, but the fecal urease produced carbon dioxide some of which was trapped as carbonate by the alkaline pH. When urine pH was decreased to from 8.5 to 6.0, carbon dioxide was dissipated, and antimicrobial effect was lost, even if the pH was re-adjusted to 8.5. *E. coli* K-12 and O157:H7 cultures that were treated with sodium carbonate (100 mM, pH 8.5, 24 h) did not persist, and dairy cattle manure (feces to urine ratio of 2.2 to 1) that was supplemented by sodium carbonate addition (8 g/kg, 5 days) had *E. coli* counts less than 10 cells per g. If the manure pH declined, the carbonate treatment was not always effective, but pH declines could be prevented by sodium hydroxide. When sodium hydroxide was included (2 g/kg), sodium carbonate additions could be decreased (4 g/kg), and treatment time was still only 5 days. Water dilution (3-fold) did not diminish the effectiveness of the carbonate/alkali treatment, and viability was still less than 10 cells per g. Based on 14,000 kg manure per cow per year, the treatment cost could be less 10 dollars per year per dairy cow.

Key Words: *Escherichia coli*, Manure, Carbonate

465 *Lactobacillus* spp. prevent *Salmonella enteritidis* colonization in chicken intestine. L.Z. Jin* and X. Zhao, *McGill University/Macdonald Campus, Quebec, Canada*.

The objective of this study was to evaluate the capability of the highly adhesive *Lactobacillus* strains to prevent the attachment of *Salmonella enteritidis* to chicken intestine. The six most adhesive *Lactobacillus* strains were isolated from chicken intestine and selected, based on their adherent ability and tolerance to low pH and bile. Arbor Acres broiler chicks were randomly assigned to three groups of 25 each. Chicks in group1 acted as control, and those in groups 2 and 3 were given a single strain of *L. acidophilus* MM8 (the most adherent among the six strains, 10^9 CFU/ml) or the mixture of the 6 *Lactobacillus* strains (10^9 CFU/ml) on day 1. On day 3, all the birds in each group were challenged with 0.5 ml of *S. enteritidis* at a concentration of 10^5 CFU/ml. Treatment of chicks with a mixture of *Lactobacillus* significantly reduced ($P < 0.05$) the mean number of *S. enteritidis* in the duodenal, ileal and cecal contents 5 and 10 days after being challenged by *S. enteritidis*. Treatment with *L. acidophilus* MM8 also significantly reduced the number of *S. enteritidis* in cecal contents 5 and 10 days after challenge. More than 90% of chicks in the control group were colonized with *S. enteritidis* in ileum and cecum while *L. acidophilus* MM8 or the mixture of *Lactobacillus* reduced the cecal colonization of *S. enteritidis* to 45% and 35%, respectively; and ileal colonization to 70% and 30%, respectively. In conclusion, the ability of *L. acidophilus* MM8 or a mixture of *Lactobacillus* to reduce *S. enteritidis* colonization in chicken intestine, together with their attaching ability highlights them as suitable strains to minimize the *Salmonella* colonization in poultry production.

Key Words: Lactobacillus, Salmonella, chicken

466 Survival of naturally occurring Escherichia coli and a streptomycin-resistant strain of E. coli K-12 (ATCC 35695) during the aging period of hard cheeses made from raw milk. Alex Teo [1] and J. Schlesser*[2], [1] *Illinois Institute of Technology, Chicago, IL,* [2] *Food and Drug Administration, NCFST, Summit-Argo, IL.*

The ability of naturally occurring Escherichia coli and a streptomycin-resistant strain of E. coli K-12 (ATCC 35695) to survive a standard cheese-manufacturing process and subsequent ripening was determined. Cheese samples made from raw milk with naturally occurring E. coli or a streptomycin-resistant strain of E. coli K-12 (ATCC 35695) were analyzed during the cheese-making process and subsequent ripening periods of 28, 60, 90 and 120 days. Presumptive levels of coliforms were monitored by using the 3-tube MPN-Lauryl Tryptose broth (LTB) method. Coliforms were then confirmed by using Brilliant Green Lactose broth (BGLB). In the 3-tube MPN method, presumptive E. coli was confirmed by using LTB-MUG. Cheese samples containing naturally occurring E. coli or the streptomycin-resistant strain of E. coli K-12 were plated and enumerated on selective media, such as MacConkey Sorbitol agar (MSA), Violet Red Bile Agar-MUG (VRBA-MUG), Biosynth Culture Medium (BCM), and Brain Heart Infusion-Streptomycin agar (BHI-strep). Naturally occurring E. coli showed a 1-2 log unit reduction from its original level in cheese made from raw milk after 60 days of aging at 7C. A further 3-4 log unit reduction in the level of the organism was observed after 180 days of aging at 7C. Streptomycin-resistant E. coli K-12 mutant was next used as a surrogate organism during the cheese-making process, and subsequent aging. Cheese made from raw milk inoculated with 10 [5] CFU per ml of streptomycin-resistant E. coli K-12 strain showed a 1-2 log unit reduction in numbers after 60 days of aging at 7C.

Key Words: Pathogenic Bacteria, Raw Milk Cheese, Aging of Cheese

467 The microbial ecology of milk powder processing via Terminal Restriction Fragment Patterns (TRFP-PCR) and the cloning and sequencing of community 16s rDNA for the identification of those TRF-fragments. A. Rife*[1], M. Pitesky[1], C. L. Kitts[2], and R. Jimenez-Flores[1], [1] *Dairy Products Technology Center,Cal Poly State University,* [2] *The Environmental Biotechnology Center, Cal Poly State University, San Louis Obispo, CA.*

The complex microbial communities present during milk powder production are difficult to characterize. While Fatty Acid Methyl Ester (FAME), Randomly Amplified Polymorphic DNA (RAPD), and biochemical analysis are ideal for species identification of cultured isolates; they have limitations in representing the diversity and evenness of the microbial communities present during milk powder production. Therefore, we investigated the efficacy of Terminal Restriction Fragment Pattern PCR (TRF-PCR), for bacterial community analysis to observe the location and time differences in the microbial ecology of milk powder production. Community 16s rDNA was extracted, amplified, fluorescently labeled, and digested (Hae III) from raw, condensed, and powdered milk samples at the beginning, middle, and end of production runs of a central California milk powder facility during the spring of 1999. Fluorescently labeled DNA fragments were then separated and detected using capillary electrophoresis coupled to a laser-induced fluorescence detector. Results showed a clear reduction in the microbial diversity between raw, condensed, and powdered milk. TRF fragments of approximately 500 base pairs were initially identified using the Ribosomal Database Project (www.cme.msu.edu/RDP). Therefore, to better identify which TRF peaks represented which organisms the amplified community 16s rDNA was cloned and sequenced. Based on sequencing, the areas of peaks corresponding to B. licheniformis, B. subtilis, and B. thermoleovarans together represented in the middle of the run 26% and end of the run 31% of the total. Among non-sporeformers there were increases in the total relative area between the middle and end of the run. These peaks were identified as Streptococcus cremoris, S. salivarius and gamma Proteobacteria. However, differences in the relative areas for sporeformers and non-sporeformers did not correlate with total mesophilic plate and aerobic endospore counts. Amplification of community DNA specifically between the evaporator and spray dryer produced random amplicons that showed as a smear (100 and 900 base-pairs) instead of a typical band of approximately 500 bp. Our experiments showed that the combination of casein protein combined with heat was responsible for this problem.

Key Words: Spores in milk powder, Rapid identification, Bacillus and TRF-PCR

468 BEHAVIOUR of *Listeria monocytogenes* in MINAS FRESCAL cheese made with lactic acid and mesophilic starter. M.C.M. Naldini and A.Y. Kuaye, *Universidade Estadual de Campinas.*

The Minas Frescal cheese is a Brazilian soft cheese, very similar to the Latin American White Cheese. The high moisture content (60%) makes it an extremely perishable product, with a short shelf-life, even under adequate refrigerated storage. The Brazilian dairy industries have recently substituted the use of starter bacteria with direct lactic acid addition to improve cheese yield and texture. However, direct acidification produces a cheese more susceptible to spoilage by contaminating microorganisms, including the pathogenic ones such as *L. monoytogenes*, which is recognized as a causative agent of severe foodborne infections. The objective of this work was to evaluate the behaviour of *L. monocytogenes* Scott A in Minas Frescal cheese manufactered by the traditional procedure with addition of mesophilic starter compared to the direct acidification with lactic acid. The cheeses were artificially inoculated with *L. monocytogenes* culture (10^9 cfu/ml) after cutting and whey drainage. Modified Oxford agar was used for *L. monocytogenes* counts and de Man, Rogosa, Sharpe agar was used for mesophilic starter counts in the cheeses after 0, 6, 12, 18, and 25 days of incubation at 5°C and 10°C. The results showed that in cheeses processed by the traditional way and stored at 5°C, the *L. monocytogenes* and starter counts remained almost unchanged, while both counts increased in the acidified cheese. At 10°C, *L. monocytogenes* and starter counts showed a slight decrease in the traditional cheese, with both counts increasing again in the acidified one. It was concluded that there was a strong inhibition of *L. monocytogenes* growth by the starter culture and therefore direct acidification shouldn't be recommended from a sanitary viewpoint.

Key Words: Minas Frescal Cheese, Acidified Cheese, *Listeria monocytogenes*

469 Properties and characterization of a monoclonal antibody for its use in screening endospores in skim milk powder. S. Fuller* and R. Jimenez-Flores, *Cal Poly State University, DPTC, San Luis Obispo, CA.*

The incidence of endospores in skim milk powder (SMP) is a problem since pasteurization, evaporation, and spray drying are ineffective in destroying them. Endospores are a risk in SMP because they are capable of germination and growth once the milk powder is rehydrated. Once vegetative, some organisms are capable of hydrolyzing lipids, casein, and/or starch, and fermenting lactose, all of these being detrimental to the quality of the SMP. Finding a rapid detection method for spores in SMP would be beneficial to screen the product industrially, and determine SMP quality. Detection of spores in SMP is currently conducted with traditional microbial plating, which is time consuming, inefficient and inaccurate. Our first objective was to determine the epitope of a monoclonal mouse IgG antibody (developed at NC State University). With this information, we could better evaluate the detection capabilities of our antibody and develop a rapid assay accordingly. Our second objective was to screen other spore forming strains from our spore collection. This collection of spore formers was specifically isolated from California SMP. Screening for cross-reactivity within this collection will measure the assay's applicability. We focused on the detection of spores utilizing this monoclonal antibody in an immunoassay and Western blot. We used protein extracts from the spores from our collection. This antibody does not present a strict specificity to *B. cereus*. Among the detected species were *Bacillus licheniformis, Bacillus pumilus* that have been proven to have high protelytic, amylolytic and lipolytic activities. Furthermore, detection of some of the most detrimental strains to milk powder was achieved using this antibody. Enzyme Linked Immuno Sorbent Assay (ELISA) was also used to confirm antibody specificity and sensitivity. The antibody on ELISA has limited sensitivity (one million spores per gram of SMP). However, if enough information is available with regards to the specific epitope on the endospore, a more efficient procedure or antibody can be obtained. A Western blot was used to determine the size of the epitope on the spore coat of *B.cereus* (positive control). The size of the epitope is approximately 25 kDa and is currently being sequenced.

Key Words: Spores in skim milk powder, Monoclonal antibody, Bacillus

470 Safety improvement of salmon using biopreservatives. H. Zuckerman*[1] and R. Ben Avraham[2], [1]*Technion, Haifa, Israel,* [2]*Milouda, Ashrat, Israel.*

Presence of *Listeria monocytogenes* in fresh, or even in cold smoked, salmon has become a major concern for the salmon processing industry, government agencies, and consumers. The application of bacteriocins to inhibit foodborn pathogens in general, and *Listeria monocytogenes* in particular is of great interest in the food industry. The efficacy of Microgard^{TM}200 (10%) w/v) combined with Novasin^{TM} (nisin) (0.2%) w/v) (Rhodia Inc. NJ) and sodium metaphosphate (10%) w/v) and Novagard^{TM} (lysozyme) (Rhodia Inc. NJ), to inhibit *Listeria monocytogenes* and to prolong shelf life of fresh salmon was evaluated. Chilled salmon were cut into 10x5 cm pieces and were randomly assigned to untreated controls and to treated samples. Treatments were carried out by dipping samples for 1 min into the respective solution, in 3 replications, control samples were dipped 1 min into water. The ratio of salmon samples to dipping solution was 1:4 (w/v). Samples were individually overwrapped with PVC film (15 micron) on polypropylene trays, and evaluated for surface pH, color, total and *Listeria monocytogenes* microbiological counts after 0,1,2,3,4 and 6 days of storage at 4°C. Samples treated with Microgard and Novasin reduced aerobic bacteria population by 2 log ($p<0.05$) and increased shelf life from 2 days to about 6 days and also, eliminated completely the growth of *Listeria monocytogenes*. Bacteriocin treatment neither affected surface pH values nor the color of the fish. Novagard was effective against *Listeria monocytogenes* but did not prolonged the shelf life of fresh salmon and affected its color.

Key Words: Safety, *Listeria monocytogenes*, Bacteriocins

471 Fate of toxigenic contaminant and nontoxigenic fungi in blue-veined cheese. S. M. El-Gindy*, *Assiut University, Egypt.*

Eight (8) types of local and imported Roquefort cheese samples were collected from different Egyptian markets and examined for its mold content. Danish and French samples contained only *P. roqueforti*. The Egyptian sampled was contaminated with *Asp. niger* and *Rhizopus nigricans*. Roquefort cheese curd was inoculated with spores of *P. roqueforti*, and/or *Asp. fumigatus* or *Asp. flavus*. The resultant ripe cheese was morphologically examined. The body, texture, flavor and mold growth and distribution in cheese were judged. The examination of various cheeses showed similarity of all cheeses to the well-known blue cheese. Some of the judges preferred the blue cheese inoculated with *Asp. fumigatus*. The present investigation revealed that cheeses were found to be free from and detectable amounts of aflatoxins. This could be attributed to certain factors such as ripening at low temperature, higher salt concentration, and higher acidity. Moreover, the addition of cheese starter may prevent the production of aflatoxin in cheese.

FORAGES AND PASTURES

472 High population corn grain silage in a dairy operation: A field demonstration. GA Brown*[1], [1]*University of Missouri Outreach and Extension, Columbia.*

The objective was to do a field demonstration to determine if there was an economic benefit of multiple plantings of high population corn grain to be harvested as silage. The silage would be forage in a NW Missouri dairy operation. The total size of the area to be planted was 2.8 hectares (ha). The plant population was estimated at 395,376 plants per ha. Normal plant population for hybrid seed corn silage is 65,000 plants per ha. The first planting was on June 8, 1999 and harvested on July 26. Plant height was 1.1 to 1.4 meters at harvest and estimated yield was 17 metric tons (t) per ha. The second planting was done on August 2. Due to dry weather conditions there was no harvest of the second planting, but samples were collected for nutritional analysis. Total seed cost per planting was 28.98 dollars at the current corn grain market price. A total estimated seed cost for hybrid seed corn for silage was 195 dollars. Samples for nutritional analysis were taken at the first planting pre and post harvest stages and 8 weeks after the second planting. The average of the samples, sample variance and sample standard deviations (Std. Dev.) of Dry Matter, Crude Protein, Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), Net Energy of Lactation (NeL) and Relative Feed Value (RFV) are in the table below. Results showed that there was an economic advantage in seed cost to the high population corn grain silage. Nutritional quality and yields may not be an advantage over hybrid seed corn silage. More research is needed under different management styles and climatic conditions to determine if there is a benefit to using high population corn grain silage as forage in dairy rations.

Key Words: Dairy Ration, Corn Silage, Economic

473 Effect of corn silage containing high oil, waxy, multileaf, or bm3 corn genetics on feed intake, milk yield, and milk composition of dairy cows. V. R. Moreira*[1], J. Jimmink[1], L. D. Satter[1], J. L. Vicini[2], and G. F. Hartnell[2], [1]*US Dairy Forage Research Center, USDA-ARS, Madison, WI,,* [2]*Monsanto Co., St. Louis, MO.*

This trial was part of a larger three-site experiment to evaluate corn silage hybrids. This part of the experiment involved 144 cows in early lactation. Following a 2-wk covariate period, 72 cows were assigned to one of six treatments for a 28-d treatment period. A second group of 72 cows followed the same protocol. The five hybrids evaluated were Cargill brown midrib 3 (BMR), Mycogen multileaf-TMF99 (TMF), Asgrow high oil RX 5888TC (HOC), Asgrow waxy RX 5888WX (WX), and Asgrow RX 5888 (Control). Diets contained 50% forage and 50% concentrate, except for one trt (BMRH) that contained 65% forage and 35% concentrate. The forage portion of the diets contained 75% corn silage and 25% alfalfa silage. Main ingredients in the concentrate were high moisture shelled corn, cotton seed, Soyplus®, and soybean meal. Diets were similar in NDF content (average: 30.5%), except for the BMRH, which was 34.5%. Dietary CP averaged 18.6%. Milk production was greatest with BMR and differed from the control, BMRH, and HOC trts ($P < .04$). FCM was greatest for the BMRH trt, reflecting the much higher fat test. Milk protein percent was lowest for the BMRH. Unreplicated estimates of silage and grain yields are presented.

Item	Control	BMR	BMRH	HOC	TMF	WX	$P <$
Milk, kg/d	34.9^{c}	36.0^{a}	34.9^{c}	35.1^{bc}	35.6^{abc}	35.8^{ab}	.04
Milk fat, %	3.29^{b}	3.15^{b}	3.77^{a}	3.29^{b}	3.15^{b}	3.31^{b}	.001
Milk CP, %	3.30^{ab}	3.27^{ab}	3.16^{d}	3.24^{c}	3.31^{a}	3.26^{bc}	.04
DMI, kg/d	21.1	21.0	21.1	21.0	21.3	21.5	NS
3.5% FCM, kg/d	33.8^{bc}	33.3^{c}	36.0^{a}	34.0^{bc}	33.1^{c}	34.6^{b}	.05
Silage yield, T/ha	21.7	21.0	...	23.0	20.9	23.9	...
Grain yield, kg/ha	11625	11050	...	10487	9524	12921	...

Key Words: Corn silage, Milk, Corn genetics

474 Effect of level of surface-spoiled silage on the nutritive value of corn silage diets. L. A. Whitlock*, T. J. Wistuba, M. K. Siefers, R. V. Pope, and K. K. Bolsen, *Kansas State University, Manhattan.*

Twelve ruminally cannulated crossbred steers were used to determine the effect of level of surface-spoiled silage on DM intake and nutrient digestibilities of whole-plant corn silage-based diets. Irrigated corn was harvested at the 80% milkline stage of maturity and chopped to a 10 mm particle length. A pilot-scale bunker silo, 0.9 m in depth, and a 2.8-m diameter AgBag® were filled with alternating loads of chopped forage. After 90 d, the bunker was sealed with a single sheet of polyethylene, and this silage was designeated "spoiled". The silage in the AgBag® was designated "normal". The four experimental diets contained 90% silage and 10% supplement (DM basis), and the proportions of silage in the diets were: a) 100% normal; b) 75% normal:25% spoiled; c) 50% normal:50% spoiled; and d) 25% normal:75% spoiled. The diets were fed once daily at 0700, and the amount fed was adjusted so that approximately 10% of the as-fed diet was in the feed bunk at the end of each 24-h period. The metabolism study consisted of two, 17-d periods,