
**ADSA-ASAS NORTHEAST SECTION
GRADUATE STUDENT
ORAL COMPETITION**

0695 Survival and growth of *Listeria monocytogenes* on queso fresco cheese stored under modified atmospheres. S. R. Barnes* and D. J. D'Amico, *University of Connecticut, Storrs.*

Cheese varieties characterized by high moisture and low acidity, such as queso fresco (QF), have been shown to support the growth of *Listeria monocytogenes* to very high levels during refrigerated storage. In addition to improving quality and extending shelf life, modified atmosphere packaging (MAP) has been used to control the growth of pathogenic microorganisms in various foods. The objective of this research was to determine the effect of five MAP conditions on the survival and growth of *L. monocytogenes* as postprocessing contaminants on QF during refrigerated storage at 7°C. To test the hypothesis that MAP affects *L. monocytogenes* growth on QF during storage when compared with conventional methods of packaging (i.e., vacuum), 25-g samples of QF were surface inoculated with an eight-strain cocktail of *L. monocytogenes* to achieve 4 log cfu/g. Following microbial attachment, individual cheeses were placed in 75- μ m high barrier pouches (nylon/ethylene vinyl alcohol/polyethylene), packaged under one of seven conditions (air, vacuum, 100% carbon dioxide [CO₂], 70% CO₂/30% nitrogen [N₂], 50% CO₂/50% N₂, 30% CO₂/70% N₂, or 100% N₂), and stored at 7°C. Samples were removed weekly through 28 d of storage for enumeration of *L. monocytogenes*. Data were analyzed using one-way ANOVA. Analyses identified overall effects of time and packaging treatment on the change in *L. monocytogenes* counts over 28 d ($P < 0.001$). *Listeria monocytogenes* populations increased rapidly on cheese packaged under air, vacuum, and 100% N₂, with counts significantly differing ($P < 0.001$) from the initial inoculum by Day 7. Changes in counts over time and counts on individual days did not differ between these treatments, with means exceeding 7 log cfu/g on Day 14 and stabilizing at >8 log cfu/g through Day 28. Treatments that incorporated CO₂ at any percentage significantly limited pathogen growth over time compared with treatments without CO₂, including air and vacuum controls ($P < 0.001$). Although pathogen growth was limited, the change in counts over 28 d in CO₂ treatments was significant ($P < 0.05$), reaching a mean of 5.0 log cfu/g. Pathogen growth during storage did not significantly differ between treatments with varying percentages of CO₂. These data demonstrate that vacuum packaging and conditions containing 100% N₂ do not impede the growth of *L. monocytogenes* on QF. However, packaging under anaerobic

modified atmospheres containing CO₂ may be a promising control for limiting *L. monocytogenes* growth on QF and other high-moisture, low-acid cheeses during cold storage.

Key Words: packaging, *Listeria monocytogenes*, cheese

0696 The effects of poor maternal nutrition on dam and offspring inflammatory status throughout gestation. A. K. Jones*, S. M. Pillai, M. L. Hoffman, K. K. McFadden, K. E. Govoni, S. A. Zinn, and S. A. Reed, *Department of Animal Science, University of Connecticut, Storrs.*

We hypothesized that poor maternal nutrition during gestation exaggerates the inflammatory status of ewes throughout gestation and that this would be reflected in the immune profile of offspring during late gestation and at parturition. Pregnant western white-faced ewes ($n = 78$) were individually housed and fed 100 (CON), 60 (RES), or 140% (OVER) of NRC requirements for TDN beginning at d 30.2 \pm 0.2 of gestation. Whole blood was collected from a subset of ewes at d 24.0 \pm 0.9 and 135.0 \pm 0.3 of gestation ($n = 4$ ewes per diet per day) and from 3 to 4 offspring per diet euthanized at d 135 of gestation or within 24 h of parturition. Whole blood RNA was isolated, and expression of 84 genes mediating inflammation was profiled using a real-time PCR array. Data were analyzed using PROC MIXED in SAS for main effects and interaction of diet and day of gestation for ewes and main effect of maternal diet for offspring with the PDIF option for mean comparisons. In ewes, regardless of diet, relative to d 24, *interleukin (IL) 17 β* ; receptors for *IL1*, *IL6*, *IL8*, *IL10 α* , and *IL10 β* ; *colony stimulating factor (CSF) 2*; *CSF3*; *tumor necrosis factor superfamily member (TNFSF) 13*; *TNFSF13 β* ; *chemokine ligand 17*; *chemokine receptor 1*; *vascular endothelial growth factor A*; and *platelet factor 4* increased 3.8-, 1.7-, 2.1-, 2.4-, 1.5-, 1.3-, 1.9-, 2.0-, 1.6-, 1.9-, 3.7-, 1.7-, 1.7-, and 2.5-fold at d 135 of gestation, respectively ($P \leq 0.05$). In contrast, *chemokine ligand 10* decreased 4.1-fold at d 135 relative to d 24 in ewes, regardless of diet ($P = 0.02$). In OVER ewes, *TNFSF4* decreased 1.5-fold compared with CON ewes ($P \leq 0.05$). *Interleukin 1 receptor antagonist (IL1RN)* increased 1.8-fold in RES ewes at d 135 compared with CON ewes at d 24 ($P \leq 0.04$). In offspring, *chemokine ligand 22* increased 2.8-fold in OVER ewes compared with CON ewes at d 135 ($P \leq 0.05$). At parturition, *interferon γ* decreased 3.0- and 3.8-fold in OVER and RES ewes, respectively, compared with CON ewes ($P \leq 0.006$). In conclusion, inflammatory progression is characteristic of advancing gestation and the increased expression of *IL1RN*, an antagonist of *IL1 α* and *IL1 β* , in RES ewes at d 135 may be a protective mechanism suppressing proinflammatory signaling. The inflammatory profile of offspring was altered by poor maternal nutrition, which may negatively affect growth and health if persistent postnatally, thereby reducing offspring productivity.

Key Words: inflammation, maternal nutrition, sheep

0697 Effects of poor maternal nutrition during gestation on offspring prenatal muscle growth.

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Poor maternal nutrition during gestation can negatively affect offspring muscle development, thereby reducing production efficiency. We previously observed that cross-sectional area (CSA) of semitendinosus muscle fibers from offspring of ewes exposed to restricted feeding or overfeeding during gestation were increased at birth and reduced at 3 mo of age. Although the negative effects of poor maternal nutrition persist into postnatal growth, it is not well known when during gestation poor maternal diet affects offspring muscle growth. We hypothesized that restricted feeding and overfeeding ewes during gestation would alter fetal muscle fiber CSA during gestation. To test this hypothesis, 78 pregnant western white-faced ewes were individually housed and fed 100 (control fed, $n = 25$), 60 (restricted fed; $n = 27$), or 140% (overfed; $n = 26$) of NRC requirements for TDN beginning at $d 30.2 \pm 0.2$ of gestation. For CSA analysis, a subset of ewes was euthanized at $d 45$ ($n = 20$) or 135 ($n = 19$) of gestation and offspring were collected ($n = 10$ to 14 per treatment per time point). Offspring from control-fed, restricted-fed, and overfed ewes are referred to as CON, RES, and OVER, respectively. From offspring, longissimus dorsi and semitendinosus muscles were excised, weighed, and frozen in optimal cutting medium. Muscle sections were cryosectioned and immunostained with wheat germ agglutinin. Images ($n = 5$ per offspring) of muscle cross-sections were taken and CSA were determined using ImageJ software. The CSA data were analyzed using the MIXED procedure of SAS, with maternal diet as the main effect. No differences ($P \geq 0.53$) due to maternal diet were observed for the BW of offspring or weight of semitendinosus or longissimus dorsi (as percent of BW) at $d 45$ or 135 . At $d 45$, longissimus dorsi CSA tended to be smaller in RES and OVER compared with CON (CON: $228.8 \pm 19.0 \mu\text{m}^2$; RES: $171.0 \pm 18.1 \mu\text{m}^2$; OVER: $183.0 \pm 16.1 \mu\text{m}^2$; $P \leq 0.07$); however, no effect of maternal diet was observed for CSA of longissimus dorsi at $d 135$ ($P = 0.48$). There was no effect of maternal diet on CSA of semitendinosus ($P \geq 0.40$) at either time point. In conclusion, during the period of primary myogenesis (approximately $d 45$ of gestation) and within 15 d of the beginning of dietary treatment, poor maternal nutrition, both restricted feeding and overfeeding, during gestation caused a reduction in offspring longissimus dorsi CSA.

Key Words: maternal nutrition, muscle, sheep

0698 Effects of citral and linalool on blood neutrophil toxicity and oxidative response in dairy cows.

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Alternative therapies to controlling and treating mastitis are being pursued to reduce potential antibacterial resistance. Certain bioactive phytochemicals extracted from plants, such as citral and linalool, have demonstrated antimicrobial activity and may serve as acceptable alternatives for conventional mastitis treatments. It is unknown how these phytochemicals may interact with bovine polymorphonuclear cells (PMN), the predominant cell type recruited during mastitis. The objective of this study was to evaluate the effects of citral and linalool on cytotoxicity and the oxidative response of bovine blood PMN in vitro. Blood was sampled from four healthy, primiparous Holstein dairy cows in mid lactation (DIM > 90). Polymorphonuclear cells were isolated and incubated for 2 h with various concentrations of citral (0, 0.1, 0.2, 0.4, 0.8 and 10 $\mu\text{L}/\text{mL}$) and linalool (0, 0.1, 1.2, 2.4, and 10 $\mu\text{L}/\text{mL}$). Cytotoxicity was measured by nonradioactive, colorimetric assay to quantify lactate dehydrogenase production. Oxidative burst response for the PMN was measured by relative chemiluminescence of reactive oxidative species production after exposure to 1.6 $\mu\text{g}/\text{mL}$ phorbol 12-myristate-13-acetate in addition to citral or linalool during incubation. Data were analyzed by ANOVA using the MIXED procedure of SAS 9.2. Each phytochemical was separately analyzed. Differences among treatments were determined using the PDIFF statements and significance was declared at $P \leq 0.05$. Citral concentrations of 0.8 and 10 $\mu\text{L}/\text{mL}$ increased PMNL cytotoxicity to 33.0 and 68.3%, respectively ($P < 0.01$), relative to the control. Oxidative burst response increased at 0.01 and 0.02 $\mu\text{L}/\text{mL}$ concentrations of citral, whereas 0.4 and 0.8 $\mu\text{L}/\text{mL}$ concentrations decreased oxidative burst ($P < 0.01$). Linalool concentrations equal to or less than 2.4 $\mu\text{L}/\text{mL}$ did not alter PMN cytotoxicity relative to the control and 10 $\mu\text{L}/\text{mL}$ increased cytotoxicity to 81.2% ($P < 0.01$). Oxidative response of PMN increased for 1.2, 2.4, and 10 $\mu\text{L}/\text{mL}$ concentrations of linalool ($P = 0.02$). In summary, citral and linalool do affect in vitro bovine blood PMN cytotoxicity and oxidative burst response. Concentrations less than 0.4 $\mu\text{L}/\text{mL}$ of citral and 2.4 $\mu\text{L}/\text{mL}$ of linalool were nontoxic to bovine blood PMN and concentrations between 0.1 to 0.2 $\mu\text{L}/\text{mL}$ citral and 1.2 to 10 $\mu\text{L}/\text{mL}$ linalool increased oxidative burst response. The use of citral and linalool as an alternative therapy for mastitis is promising as they may not interfere with the immune response during mastitis.

Key Words: mastitis, polymorphonuclear cell, alternative therapy

0699 In vitro screening of the anthelmintic efficacy of birdsfoot trefoil commercial varieties and cultivars against ovine *Haemonchus contortus*.

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Some forages containing condensed tannins (CT), also called proanthocyanidins (PAC), suppress gastrointestinal nematode (GIN) infections in small ruminants. The objective of this study was to investigate the anthelmintic potential of 51 commercial varieties and cultivars of birdsfoot trefoil (BFT) against *Haemonchus contortus*. The antiparasitic activity of BFT proanthocyanidin extract (BFT-PAC) and BFT aqueous extract (BFT-AqE) was tested using the following in vitro assays: 1) egg hatching and viability of L1 *H. contortus* larvae and 2) exsheathment of L3 *H. contortus* larvae. Birdsfoot trefoil powder of each variety or cultivar was analyzed for CT content (mg/g) by the 4-(dimethylamino)cinnamaldehyde method. Birdsfoot trefoil proanthocyanidin extract was prepared by isolating PAC extract from the BFT powder using solid-phase chromatography. Birdsfoot trefoil aqueous extract was prepared by soaking BFT powder in water at room temperature for 24 h. The plant matter was then removed, leaving an aqueous extract. 1) For in vitro egg hatch and viability of L1 larvae, *H. contortus* eggs were isolated from fresh feces and exposed to varying concentrations of BFT extracts for 24 h. The percentage of hatched eggs and L1 larval mortality (based on motility) were determined. 2) For in vitro exsheathment, 2,000 *H. contortus* L3 larvae were incubated in varying concentrations of BFT extracts for 24 h followed by exsheathment using CO₂. The percentage of exsheathed larvae (based on absence of sheath) was determined. Condensed tannin content ranged between 1.5 and 63.8 mg/g across 51 varieties and cultivars. Inhibition of egg hatch and larval mortality was observed with incubation in BFT-AqE; however, the concentration at which this inhibition and mortality was most effective varied among varieties and cultivars: at 3 mg/mL, percent inhibition of egg hatch and L1 mortality spanned between 0 and 100% across 51 varieties and cultivars tested. Results for incubation in BFT-PAC and results for exsheathment are pending. Preliminary results indicate that commercial varieties and cultivars of BFT-AqE inhibited egg hatch and increased larval mortality, but the degree of inhibition and mortality varied. Additional results testing BFT-PAC and testing exsheathment will provide further information about the anthelmintic efficacy of commercial varieties and cultivars of BFT for small ruminant GIN control.

Key Words: small ruminant, sheep, condensed tannin

ADSA DAIRY FOODS GRADUATE STUDENT ORAL COMPETITION

0700 Anti-obesity and antidiabetic properties of lactoferrin are independent of calorie intake.

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Whey proteins provide multiple health benefits to humans including promotion of weight loss and improving diabetic control. However, the bioactive components of whey that produce such benefits and the underlying mechanisms of action are poorly understood. Our objectives were to determine the effects of whey, and its components lactalbumin and lactoferrin, on 1) energy balance, body composition, glucose tolerance, and gut hormones and 2) key regulatory markers of glucose and lipid metabolism in liver and skeletal muscle of diet-induced obese (DIO) rats. The DIO rats were randomized to receive one of 5 isocaloric dietary treatments ($n = 8/\text{group}$; 40% fat and 4.63 kcal/g)—control (CON; 15% protein), whey (WH), lactalbumin (LA), lactoferrin (LF), and pair-fed WH to LF (PF)—for approximately 8 wk. The high-protein diets contained 15% added whey or its components. Food intake, meal patterns, energy expenditure, body composition, glucose tolerance, plasma hormone, and hepatic and muscle mRNA abundance were measured. Data were analyzed by linear mixed models, ANOVA, or ANCOVA. We found that 1) compared with CON, WH, LA, and LF reduced food intake, with LF producing a greater and sustained reduction of intake; 2) the hypophagia is partly due to reduced meal size and/or frequency, increased peptide YY mediated satiety, and decreased diet preference; 3) LF produced greater reductions in BW and fat mass, enhancement in energy expenditure, and improvement in glucose tolerance than PF; 4) LA decreased BW and fat mass, increased energy expenditure, and improved glucose tolerance compared with CON; 5) LA and LF decreased plasma concentrations of insulin and leptin relative to CON; and 6) LA increased the mRNA abundance of GLUT2, glucokinase, glycogen synthase, and carnitine palmitoyltransferase-1 and decreased fatty acid synthase and pyruvate dehydrogenase, whereas LF increased glucokinase and glucose-6-phosphate dehydrogenase and decreased phosphofructokinase and fatty acid synthase in the liver and both LA and LF increased muscle pyruvate dehydrogenase compared with CON. Together we demonstrate that the improvement in energy balance, lipid metabolism, and glucose tolerance by lactoferrin are beyond its hypophagic effects. Our findings have important implications for developing lactalbumin- and lactoferrin-based functional foods and nutraceuticals for weight loss and diabetic control. Funding by ALMA, AI-Bio, and Alberta Milk.

Key Words: diabetes, lactalbumin, lactoferrin, obesity, whey