

of PEMF on circulating inflammatory markers in healthy lactating dairy cows. Mid-lactation, organic Jersey cows ($n = 36$) of various parities were randomly assigned to 1 of 3 treatments (TRT): 1) control (CON; $n = 12$); no PEMF therapy, 2) once daily PEMF (PEMF1; $n = 12$), or 3) twice daily PEMF (PEMF2; $n = 12$). Treatment consisted of 8 min of PEMF on the topline, followed by 4 min of PEMF on each udder quarter, and was administered immediately after the AM (PEMF1 and PEMF2) and PM (PEMF2) milkings. Cows were enrolled on the study for 10 consecutive days; 2 d of baseline (BL) where no treatment was given, 3 d of treatment (TX), and 5 d of follow-up (FU). Composite milk samples were collected twice daily during all study periods and blood samples were collected at d 1 of BL, d 2 and 3 of TX, and d 2 of FU. Serum was analyzed for albumin (ALB) and serum amyloid A (SAA). Only 25% of samples exceeded the reference threshold for SAA (>5 g/dL), thus was not statistically analyzed. Mixed linear models were created to assess the difference in ALB between TRT groups for BL, TX, and FU periods. Study day, when appropriate, and TRT were retained in all models regardless of significance. For all study periods, ALB was similar between TRT groups (all $P > 0.1$). Parity differences existed for all study periods (all $P < 0.05$), where primiparous cows (BL: 3.94 [95% CI: $3.87, 4.82$] g/dL; TX: 3.96 [3.89, 4.04] g/dL, FU: 3.91 [3.84, 3.99] g/dL) had greater ALB concentrations than multiparous cows (BL: 3.79 [3.76, 3.86] g/dL; TX: 3.79 [3.72, 3.87] g/dL; FU: 3.77 [3.69, 3.84] g/dL). Although we saw no effects of PEMF on inflammation, further investigations of cows with inflammation are needed.

Key Words: pulsed electromagnetic field therapy, inflammation, organic dairy

2336 Early lactation health and milking behavior in cows under an automatic milking system. C. Hernandez-Gotelli¹, D. Manriquez², and P. Pinedo^{*1}, ¹Colorado State University, Fort Collins, CO, ²AgNext, Colorado State University, Fort Collins, CO.

Automatic milking systems (AMS) collect abundant cow-level information that could be used to detect behavioral changes that identify sick cows. The objective was to examine the associations of milking behavior and performance variables with early lactation diseases in cows using an AMS. This study was conducted on a commercial dairy operation in Colorado. The data included 5,222 lactations of multiparous Holstein cows that calved between January 2023 and April 2024 and were milked using 62 AMS. Milking behavior and performance data included milk yield (kg), milking duration (min), milking interval (h), milk flow duration (sec), mean milk flow (kg/min), and the frequency of teat not found, milking unit kick-off, and incomplete milking events for every AMS visit. Health disorders from 0 to 21 DIM, included clinical hypocalcemia (HCa), metritis, mastitis, and displaced abomasum (DA). A healthy group was used as a reference for comparisons. Milking behavior and performance were examined from 1 to 21 DIM for sick and healthy cows. Multivariable mixed linear and logistic regression models for repeated measures were fit using milking behavior and performance variables as the responses of interest. Lactation number and milk yield (when appropriate) were included as fixed covariates, and cow ID was considered a random effect. Compared with healthy cows, milk yield was lower in cows with HCa, metritis, mastitis, and DA. Cows that developed metritis, mastitis, and DA had longer milking durations than healthy cows. Longer milking intervals were identified for all the diseases in analysis. Cows affected by metritis, mastitis, and DA had longer milk flow duration, whereas cows with metritis and mastitis had lower mean milk flow compared with healthy controls. The probabilities of incomplete milking and teat not found were greater only for mastitic cows. As anticipated, health disorders associated with pain

or discomfort significantly affected milking behavior and performance during early lactation. In conclusion, differences in milking behavior and performance between sick and healthy cows were established for most of the disorders in analysis. Integrating changes across various milking variables can enhance the early detection of diseases in AMS.

Key Words: milking behavior, robotic milking, health

2337 Impact of heat stress on intramammary *Escherichia coli* challenge in lactating Girolando cows: Effects on body temperature, plasma insulin, cortisol, and milk yield. A. S. Silva^{*1}, J. Diavão¹, L. C. Mendonça¹, M. M. Campos¹, W. A. Carvalho¹, and J. A. Negrão², ¹Empresa Brasileira de Pesquisa Agropecuária - EMBRAPA Gado de leite, Juiz de Fora, Minas Gerais, Brazil, ²Faculdade de Zootecnia e Engenharia de Alimentos (FZEA), Universidade de São Paulo (USP), Pirassununga, São Paulo, Brazil.

This study aimed to evaluate the effects of lipopolysaccharide of *Escherichia coli* (LPS) challenge under heat-stressed cows on vaginal (VT; iButton, Whitewater, WI), eye, and udder thermal temperatures (T420; FLIR Systems Inc., Wilsonville, OR), plasma insulin, cortisol and milk yield. Twenty-four Girolando cows (3/4 Holstein \times 1/4 Gir, with 111 ± 54 DIM, and 19.4 ± 4.5 kg/d of milk yield) were randomly assigned in a block design with 2 treatments: control (CT) and heat stress (HS). The CT cows remained under a cooling system in freestall (temperature-humidity index [THI] of 72) and HS cows were submitted to a THI of 84 in a climatic chamber from 0700 to 1600 h, for 13 d. All cows were submitted on the 10th day to a single dose of LPS challenge in the left rear udder (100 μ g of LPS diluted into 10 mL of saline solution 0.9% NaCl) and received 10 mL of saline solution (0.9% NaCl) in the right rear udder. The VT was measured every hour during the LPS challenge (from 10 to 13 d); blood samples were collected at -24, 0, 4, 6, 10, 24, 48, 72 h relative to the LPS challenge; and eye and udder temperatures were measured at 0, 1, 4, 6, 24, 48, and 72 h of LPS challenge. Data were analyzed using a complete block design (SAS Institute Inc.) with repeated measures. Significance was considered when $P \leq 0.05$. The HS cows had greater VT during the LPS (HS = 39.1 vs. CT = 38.7°C ; $P = 0.01$). Heat-stressed cows had greater eye (HS = 39.1 vs. CT = 38.3°C), right rear udder (HS = 38.7 vs. CT = 37.7°C), and left rear udder (HS = 38.7 vs. CT = 37.9°C), temperatures at 4 h ($P < 0.05$). Cortisol was greater only at 12 h after the LPS in HS cows (HS = 107.8 vs. CT = 86.2 ng/mL; $P = 0.01$); insulin increased in HS cows only at 12 h after the LPS (HS = 1.87 vs. CT = 1.09 ng/mL) and 24 h after the LPS (HS = 2.10 vs. CT = 1.21 ng/mL; $P = 0.04$). Milk yield was not influenced by heat stress during LPS challenge (HS = 15.0 vs. CT = 15.6 kg/d; $P = 0.24$). Although HS cows exhibited greater body temperatures during the LPS challenge, along with increased cortisol and insulin, HS cows did not decrease milk yield during LPS challenge.

Key Words: cortisol, immune response, tropical climate

2338 Effect of intra-mammary infusion of a carvacrol-based botanical product at dry-off on udder health. P. Munoz-Boettcher^{*1}, C. Hernandez-Gotelli¹, C. Ibarguren¹, R. W. Zheng¹, J. Velez², A. De Vries³, E. K. Miller-Cushon³, B. J. Heins⁴, R. A. Lynch⁵, Q. Kolar⁵, G. M. Schuenemann⁶, V. E. Cabrera⁷, E. Silva⁷, and P. Pinedo¹, ¹Colorado State University, Fort Collins, CO, ²Aurora Organic Farms, Platteville, CO, ³University of Florida, Gainesville, FL, ⁴University of Minnesota, Morris, MN, ⁵Cornell University, Ithaca, NY, ⁶The Ohio State University, Columbus, OH, ⁷University of Wisconsin, Madison, WI.

Table 1 (Abstr. 2338). Distribution (%) of the main bacteriological isolates found at dry-off and during the first (W1) and second (W2) week postpartum by dry-off treatment¹

Item	Dry-off (n = 203)		W1 (n = 155)		W2 (n = 151)	
	CON	BT	CON	BT	CON	BT
No growth	40.0	46.2	54.5	59.3	48.6	60.5
<i>Staphylococcus aureus</i>	20.0	17.3	18.2	19.8	23.0	27.6
Non- <i>aureus</i> staphylococci	34.3	28.8	22.1	12.3	21.6	2.6
<i>Escherichia coli</i>	2.9	1.0	1.3	1.2	0.0	0.0
Other	1.9	3.8	3.9	4.9	4.1	9.2
Contaminated	1.0	2.9	0.0	2.5	2.7	0.0

¹No statistically significant differences between CON and BT were identified.

The objective was to evaluate the effect of intra-mammary infusion of a carvacrol-based botanical product, formulated to maintain or restore the normal uterine environment, on udder health in the subsequent lactation of Holstein cows managed in an organic system. A total of 203 cows were enrolled at the time of dry-off and were assigned randomly into 1 of 2 treatment groups: (1) control (CON; n = 101; average parity = 3.37) receiving 10 mL of distilled water in each quarter and (2) botanical therapy (BT; n = 102; average parity = 3.16), receiving 10 mL of an intramammary infusion of Optimum UterFlush (Van Beek Natural Science, Orange City, IA), diluting one syringe of 30 mL in 1,000 mL of distilled water. Composite milk samples for bacteriological culture and SCC were collected at dry-off (DO) and during wk 1 (W1) and wk 2 (W2) postpartum. Outcomes of interest consisted of SCC and bacterial infection at W1 and W2 and clinical mastitis and culling within the first 30 DIM. Data were analyzed using logistic regression by univariable models followed by multivariable models considering parity number, days dry, and bacterial growth and SCC at DO as covariables. Table 1 shows the pathogen distribution by sampling time. No treatment effect on the probability of a SCS <4 (200,000 SCC/mL) on W1 and W2 was observed. There was no treatment effect on the distribution of cultured pathogens at W1 and W2 when no growth versus growth, gram-positive versus gram-negative, and major versus minor pathogen categories were compared. No differences were observed between treatment groups for clinical mastitis or culling within the first 30 DIM. In conclusion, no effects on udder health, for parameters assessed, were identified following an intra-mammary infusion at dry-off of the botanical product analyzed in this trial.

Key Words: mastitis, organic, dry-off

2339 Molecular characterization and resistance modulation strategies against methicillin-resistant *Staphylococcus aureus* from cattle mastitis. M. Ijaz*, M. U. Javed, A. Ahmed, H. Rasheed, and A. A. Jabir, *University of Veterinary And Animal Sciences, Lahore, Punjab, Pakistan.*

The emergence of antimicrobial resistance (AMR) poses a major challenge, reducing the range of therapeutic choices for both veterinary and public health professionals. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important antimicrobial-resistant strains of *S. aureus* for causing subclinical mastitis in cattle around the globe. For this purpose, 384 milk samples from the lactating cattle were collected using convenient sampling techniques. The samples were initially screened for subclinical mastitis (SCM) by using the California mastitis test. The SCM-positive samples proceeded to standard microbiological procedures for the isolation of *Staphylococcus aureus*. The *S. aureus* isolates were confirmed as MRSA based on the cefoxitin disk diffusion test and the presence of the *mecA* gene by PCR followed by

phylogenetic analysis using bioinformatics tools. The prevalence was determined using the formula described by Thrusfield, whereas the categorical data for antimicrobial susceptibility tests were analyzed as percentages through descriptive statistics. The results revealed that out of 384 milk samples, 233/384 (60.68%) samples were positive for subclinical mastitis, whereas the prevalence of *S. aureus* was noted to be 29.42%. The phenotypic and genotypic prevalence of MRSA was noted to be 41.59% and 24.78%, respectively. The findings of the phylogenetic analysis of the *mecA* gene revealed a strong evolutionary connection between the study isolates and MRSA isolates of Pakistani cattle and buffalo of different studies. Antimicrobial susceptibility testing of MRSA isolates exhibited the highest resistance to penicillin, followed by gentamicin and oxytetracycline, whereas moxifloxacin and linezolid were among the susceptible antibiotics. Synergy testing using the checkerboard assay demonstrated a synergistic effect between penicillin and ketoprofen, gentamicin and flunixin meglumine, and oxytetracycline with *N*-acetylcysteine. The current study concluded that MRSA is an emerging and prevailing issue associated with subclinical mastitis in cattle and the use of antibiotics in combination with non-antibiotics as potential therapeutic agents for resistance modulation against MRSA. This study will help to devise treatment and control strategies against cattle mastitis

Key Words: antimicrobial resistance (AMR), methicillin-resistant *Staphylococcus aureus* (MRSA), *mecA*, cattle, phylogenetic analysis

2340 Control of spinose ear tick in dairy cows with permethrin spray. P. Jardon*, A. Rico^{2,3}, A. Gerry⁴, B. Karle⁵, and N. Silva-del-Rio^{2,3}, ¹Iowa State University, College of Veterinary Medicine, Ames, IA, ²UC Davis School of Veterinary Medicine, Population Health and Reproduction, Davis, CA, ³Veterinary Teaching and Research Center, Tulare, CA, ⁴University of California Riverside, Entomology, Riverside, CA, ⁵University of California Agriculture and Natural Resources, Davis, CA.

The objective of the present study was to evaluate the effectiveness of a 0.50% permethrin spray (Catron IV, Elanco Animal Health) in reducing spinose ear tick (*Otobius megnini*) infestation in lactating dairy cows and its impact on milk yield. The trial was conducted on a 2,400-cow freestall commercial dairy in California with a known spinose ear tick infestation. The study was implemented from October to November 2024. A total of 647 Holstein cows that met the selection criteria (90–180 DIM, ≥35 kg daily milk yield, and ≤500,000 somatic cell count) were enrolled. Eligible cows were housed in 2 different pens and randomly allocated within pen to either control (CON; no treatment, n = 300) or tick treatment (TT; n = 347) group based on the odd/even number of their ear tag ID. Cows assigned to treatment group received ≈3 mL application of 0.5% permethrin spray inside each ear at study initiation and again 15 d later. Researchers evaluated tick infestation weekly using a 4-point tick score (TSc: 0 = no ticks, TSc: 1 = 1–5 ticks, TSc: 2 = 6–10 ticks, TSc: 3 = >10 ticks); both ears were assessed, and the highest score from either ear was recorded as the score for that animal. Daily milk yield data were collected for 4 weeks, and changes in weekly milk yield averages were analyzed. An ordinal logistic mixed model was used to assess treatment effects on TSc, whereas a linear mixed model evaluated treatment effects in weekly milk yield. At enrollment, no significant differences were observed between groups in parity ($P = 0.97$), weekly milk yield ($P = 0.15$), DIM ($P = 0.12$), or tick infestation scores ($P = 0.39$). A significant treatment-by-week interaction ($P < 0.01$) was observed. By wk 3, the distribution of TSc 0, 1, 2, and 3 was 3.6%, 3.2%, 11.7%, and 81.4% in CON vs. 80.5%, 18.0%, 1.5%, and 0% in TT, respectively. However, no significant difference was observed