Monday June 26th: Production, Management, and the Environment 1

Effects of heat stress and supplementation of rumen-protected methionine during the transition period on immune function and liver functionality index of Holstein cows

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ORAL 2181

Exposure to heat stress during the transition period is associated with impairment of immune function. Feeding rumen-protected Met (RPM) during the transition period improves immune function. Therefore, we aimed to evaluate the effects of supplementing RPM to Holstein cows under heat stress during the transition period on immune function and liver functionality index (LFI), which assesses transition cow's metabolic health. Cows blocked by parity and ME305 were assigned to thermoneutral conditions (TN, avg THI 63, n=19), heat stress induced by electric heat blankets (HS, n=17), and HS with inclusion of RPM in the total mixed ration (MHS, 0.1% of DM of Smartamine®M Adisseo Inc., Antony, France, n=17) for 4 weeks pre- and 4 weeks post-calving. Blood samples were collected at 1, 8, and 28 days in milk (DIM) for hemogram, leukogram, albumin, cholesterol, and total bilirubin quantification, and granulocyte's phagocytosis and oxidative burst analyses. Data were analyzed using generalized linear mixed models in SAS, considering the fixed effects of treatment (TN, HS, MHS), DIM, and block. Contrasts included C1 (TN vs average of HS and MHS) and C2 (HS vs MHS). Cows in TN had greater hematocrit and lower neutrophil count (C1 P=0.04; 31.3±0.69% and 2.27±0.30×109 cells/L) than cows in HS and MHS (29.8±0.69% and 2.83±0.30×109 cells/L). Cows in TN had greater granulocyte's phagocytosis percentage (C1 P=0.01; 47.1±9.67%) than HS and MHS cows (17.4±9.67%). Cows in HS had lower albumin concentration at 28 DIM (C2 P=0.03; 26.3±2.04 g/dL) than cows in MHS (32.3±2.04 g/dL). There was a lower (P=0.01) proportion of cows classified as high LFI in HS (18%, 2/11) than in TN (75%, 12/16) and MHS (60%, 9/15). Finally, the odds of being classified as high LFI were lower in HS than in MHS (OR=0.15, P=0.06) and TN cows (OR=0.07, P=0.01). In conclusion, HS induced with heat blankets alters immune function mainly related to neutrophils' count and phagocytosis activity. Feeding RPM to transition cows under HS improved LFI, but effects on immune cells and granulocyte's function are limited.

Adisseo Message:

As climate change occurs, heat stress will continue to cause losses for the livestock industry. Supplemental methionine during the peripartum period has been increasing on commercial farms due to the beneficial effect of methionine on milk production, immune function, inflammation, and metabolism. This study aimed to further understand the beneficial effects of methionine during the peripartum period by examining the effects of rumen-protected methionine supplementation during exposure to heat stress during the peripartum period. The results underscore the negative impacts of heat stress on immune function and liver function. However, supplying methionine to peripartum cows under heat stress improved liver function, which can help the cow to better handle the negative metabolic effects associated with heat stress.

Monday June 26th: Ruminant Nutrition 2 - Protein and Amino Acids

Amino acid supplementation as a potential strategy to mitigate milk fat depression

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ORAL 2208

In dairy cows, a shift in rumen biohydrogenation of dietary polyunsaturated fatty acids (PUFA) leads to milk fat depression (MFD). The objective of this study was to determine the effect of increasing metabolizable Met and Leu supply on milk fat production in control (high-palmitate fat source) and MFD-induced (soy oil) cows. The study was designed as a replicated 4x4 Latin square with four 28-d periods and 4 treatments arranged as a 2x2 factorial. All diets included high starch (28%). Factors were fat source (FS): soybean oil (SBO; 1.83% DM) or a 80% palmitate fat supplement (CTL; 1.89% DM); and AA level (AAL), either 5% metabolizable protein deficient (AAD), or sufficient, balanced for Leu and Met with corn gluten meal and rumen protected Met (AAS). Fifty-six Holstein cows (71 DIM), housed in tie stalls and fed 1x/d and milked 2x/d were enrolled as two cohorts and grouped within cohort by DIM into 14 squares. Cows were weighed weekly, and DMI and milk yield and composition were measured on d 23-26 of each period. Data were analyzed with a mixed model containing FS, AAL, FSxAAL, period, and square within cohort as fixed effects and cow within square as random. There was no significant interaction between factors for any reported variables. Body weight change was not affected by either factor ($P>0.10$). Dry matter intake was not affected by FS ($P=0.52$) but was decreased by AAS (-1.6 kg/d, $P<0.001$). Milk and lactose yield were not affected by either factor ($P>0.21$). As expected, compared to CTL, SBO decreased ($P<0.001$) fat yield (-150 g/d) and percent (3.32, 3.69 $\pm$ 0.06%), and AAS tended to increase fat yield compared to AAD (+43 g/d, $P=0.09$), but not percent (3.54, 3.48 $\pm$ 0.06%; $P=0.30$). Protein yield was not affected by fat source ($P=0.44$) but was increased by AAS (+69 g/d, $P<0.001$). Milk urea nitrogen decreased with SBO vs CTL (13.0 vs 13.7 mg/dL, $P=0.01$) and tended to increase with AAS vs AAD (13.6 vs 13.1 mg/dL, $P=0.08$). Overall, AA supplementation tended to increase milk fat yield; however, the effect of AA supplementation was not sufficient to overcome the differences in milk fat production between dietary fat sources.

Keywords: Milk fat depression, amino acids

Adisseo Message:

Methionine and leucine are two amino acids (AA) that stimulate the mechanistic target of rapamycin complex 1 (mTORC1), a master regulator of nutrient metabolism that controls milk protein and fat synthesis. In line with this, methionine and leucine supplementation has been shown to increase milk and milk components production. This study was designed to evaluate if balancing lactation diets for those AA could mitigate the deleterious effects of milk fat depression induced via dietary polyunsaturated fatty acids. As expected, supplying dietary soybean oil (linoleic acid) decreased milk fat percentage and yield compared to the control (palmitate fat supplement). While AA supplementation was not able to completely overcome the milk fat depression induced by soybean oil, it increased milk fat content and tended to increase milk fat production under both fat sources, partially mitigating the milk fat depression. These results further underscore the beneficial effects of AA supplementation on increasing milk fat production.

Monday June 26th: Ruminant Nutrition - Protein and Amino Acids 1

Energy source and amino acids independently alter mammary extraction of nutrients

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POSTER 1249M

The objective of this study was to determine if metabolizable AA level (AAL) altered mammary extraction of nutrients based on energy source (ES, glucogenic (GE) vs ketogenic (KE)). Twenty dairy cows (75 DIM) were enrolled in a replicated 4x4 Latin square with four 28-d periods and 4 treatments arranged as a 2x2 factorial. Factors were AAL: 10% metabolizable AA deficient (DAA) or sufficient, balanced for Met, Lys, and Leu (BAA); and ES: GE (29.5% starch, 3.5% fat) or KE (21% starch, 6% fat, soy hulls replacing corn). Blood samples were collected from a coccygeal vessel and the subcutaneous abdominal vein, 6 times across d25-d26 of each period, staggered to represent every 2 h sampling between am and pm milkings. Plasma was analyzed for insulin, glucose, beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), triacylglycerol (TG), and AA. Data were analyzed with a mixed model containing ES, AAL, ESxAAL, period, and square as fixed effects and cow within square as random. Mammary plasma flow, based on the Fick principle, was not affected by ES (P=0.25) but tended to decrease in response to BAA (-54 L/h, P=0.13). Plasma insulin was higher for GE than KE (0.60, 0.54 ug/L P=0.02) and increased more in response to BAA under GE than under KE (0.16, 0.06 ug/L P=0.03). Plasma concentration and mammary extraction and uptake of glucose were not affected by ES or AAL (P>0.15). Plasma concentration of BHB, NEFA, and TG increased by KE (P<0.01) but were not affected by BAA (P>0.15). Mammary extraction and uptake of TG were increased (P<0.001) by KE, while BAA increased mammary extraction of BHB (+5%, P<0.001) and uptake of NEFA (+9.2%, P=0.01). Plasma concentration of essential AA (EAA), except Phe and Thr, increased for KE vs GE ($P\leq$0.01), but mammary uptake on KE increased only for Arg (P=0.01) and decreased for Leu (P=0.02). Plasma concentration of His, Leu, Lys, Phe, and Val increased (P<0.001) and Thr and Trp decreased (P<0.001) for BAA. Mammary uptake of EAA increased (P<0.01) for BAA, except for Arg (P =0.3) and Trp that tended to increase (*P*=0.13). In conclusion, there was no interaction between ES and AAL on mammary uptake of individual nutrients.

Keywords: Mammary gland, energy, amino acids

Adisseo Message:

Amino acid balancing of dairy cow rations has been observed to enhance both milk protein and fat synthesis. Additionally, insulin and glucogenic energy have been observed to stimulate milk protein yields in dairy cows. Both insulin and amino acids (AA), particularly methionine and leucine, stimulate the mechanistic target of rapamycin complex 1 (mTORC1), a cellular nutrient sensing complex that regulates metabolic processes like protein and fat synthesis. Therefore, the objective of this trial was to determine if mammary extraction of nutrients for the synthesis of milk components is affected by energy source (glucogenic or ketogenic) and a balanced AA supplementation.

Overall, energy source did not affect mammary extraction of nutrients, except fatty acids (FA) that were supplied at a higher level by the ketogenic diet. On the other hand, balanced AA supplementation increased mammary extraction of AA and FA, in line with the observed response in milk protein and fat production by that treatment. The observed results underscore the benefits of AA balancing on mammary uptake of essential AA, which can support the activation of mTORC1 and, thereby, milk components synthesis.

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Wednesday June 28th: Ruminant Nutrition: Protein and Amino Acids 3

Effect of rumen-protected methionine supplementation in a low starch diet with or without supplemental sugar on the productive performance of dairy cows.

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POSTER 1788W

The main objective of this study was to evaluate the effect of rumen-protected methionine (RPM) supplementation in a low-starch diet, with or without an extra source of sugar, on the productive performance of mid-lactation dairy cows, fed with isoproteic diets based on corn and ryegrass silage, dry ground corn and soybean meal. Eighty multiparous Holstein cows with 2.4 ± 0.6 lactations, 36.5 ± 4.5 kg/d, and 160 ± 68 DIM, were randomly distributed in a 2 x 2 factorial arrangement. Cows were blocked by parity, milk yield, and DIM, and allocated in 4 treatments: CON (control diet with 23% starch (%DM) without sugar supplementation or RPM); MET (control diet with RPM supplementation; 14 g of Smartamine M®, Adisseo); SUG (control diet with 1.7% DM of crystal sugar); and MET+SUG (the inclusion of both treatments). The total experimental period was 28 d, with the first 3 d used for data collection as covariates. A TMR was provided, and treatments were top-dressed once a day. Milk yield and composition were recorded weekly and in the last 9 milkings. Data were analyzed using the MIXED procedure of SAS containing the fixed effects of MET, SUG, parity, time, and their interactions, and the random effects of block and cow. Multiple treatment comparisons were made among treatments and corrected using a Tukey adjustment. The inclusion of both sugar treatments (SUG and MET+SUG) increased milk yield and the inclusion of both RPM treatments (MET and MET+SUG) increased milk protein content.

Table 1. Effects of methionine and sugar supplementation on productive performance.

Variables	CON	MET	SUG	MET+ SUG	SEM	MET	SUG	MET* SUG
Met (%MP)	2.10	2.40	2.10	2.40				
Lys:Met	3.06	2.68	3.06	2.70				
Met:ME	0.91	1.04	0.91	1.04				
Milk yield, kg/d	34.20[b]	32.70[c]	35.40[a]	35.10[a]	0.28	0.33	<0.01	0.03
ECM, kg/d	35.00	33.90	36.20	36.00	0.29	0.69	<0.01	0.06
Fat, %	3.55	3.89	3.76	3.77	0.04	0.03	0.03	1.00
Total protein, %	3.35[b]	3.46[a]	3.39[b]	3.43[a]	0.02	<0.01	0.68	0.02
Lactose, %	4.56	4.51	4.65	4.51	0.02	<0.01	0.22	0.49
Fat, kg/d	1.23	1.25	1.32	1.31	0.01	0.57	0.08	0.34
Total protein, kg/d	1.16	1.12	1.19	1.19	0.01	0.83	0.01	0.06
Lactose, kg/d	1.56	1.48	1.65	1.59	0.02	0.03	<0.01	0.15
MUN, mg/dL	22.55	23.56	23.99	22.09	0.29	0.55	0.48	0.78

Key Words: amino acid, energy, sugar

Adisseo Message:

The results from this trial suggest that despite the basal diet being supplemented with enough Smartamine M to closely meet the recommended grams of metabolizable Met:ME ratio, the total energy, of the diet may have not been sufficient to elicit a milk volume response with the added methionine coming from Smartamine M. Although total milk protein was not increased, it is noteworthy that milk protein percentage increased with added methionine.

The added sugar did elicit a milk volume response, therefore, supporting the assessment that the basal diet was energy limiting. In agreement with previous reports (Boderick et al., 2008), adding crystal sugar did not lower the MUN.

Before adding Smartamine M to diets that are known to be deficient in methionine, the end user needs to assure that the basal diet carries enough ME to capture the benefits of the additional methionine.

Tuesday June 27th: Ruminant Nutrition: Protein and Amino Acids 2

Feeding rumen-protected methionine and calcium salts enriched in omega-3 fatty acids increase fatty acid and methionine intakes, increase plasma methionine concentrations, and alter milk fatty acid profiles in periparturient dairy cows.

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POSTER 1527T

The objective of this study was to investigate the effects of feeding rumen-protected (RP)-Met and calcium salts (CS) of fatty acids (FA) enriched without or with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA; i.e., n3FA) in periparturient cows. In a randomized complete block study design, 75 multiparous Holstein cows were assigned to 1 of 4 treatments (n = 18-19/diet): 1) Met deficient (-Met) with CS not enriched in n3FA (-n3FA; 0% n3FA; EnerGII; Virtus Nutrition, USA), 2) Met adequate (+Met; Smartamine M; Adisseo Inc., France) with -n3FA, 3) -Met with CS enriched in n3FA (+n3FA; 4% n3FA; EnerG-3; Virtus Nutrition), or 4) +Met with +n3FA from wk -3 prior to expected calving through wk 4 of lactation. Cows were fed corn silage-based total mixed rations, pre- and postpartum, which were formulated to provide Met at ≤0.96 or ≥1.13 g per Mcal metabolizable energy (ME) for -Met and +Met, respectively. CS were fed at 1.5% ration dry matter pre- and postpartum. Feed samples were collected weekly. Milk samples were collected twice weekly following a thrice daily milking schedule. Pre- and postpartum data were analyzed separately using PROC MIXED of SASv9.4. Planned contrasts included: 1) effect of Met (-Met vs. +Met), 2) effect of n3FA (-n3FA vs. +n3FA), and 3) effect of co-supplementation (+Met/+n3FA vs. +Met/-n3FA and -Met/+n3FA). Pre- and postpartum Met intakes (g Met/Mcal ME) were greater in +Met/+n3FA ($P < 0.001$), relative to +Met/-n3FA and -Met/+n3FA. Intakes of EPA and DHA in both pre- and postpartum cows were greater in +Met/+n3FA ($P < 0.001$), relative to +Met/-n3FA and -Met/-n3FA. Milk EPA and DHA percentages were greater in +Met/+n3FA, relative to +Met/-n3FA and -Met/+n3FA ($P \leq 0.01$). Similarly, milk EPA and DHA yields were greater in +Met/+n3FA, relative to +Met/-n3FA and -Met/+n3FA ($P \leq 0.001$). In conclusion, feeding transition cows RP-Met and CS enriched in n3FA altered amino acid and fatty acid intake and increased milk n3FA percentages and yields

Keywords: methionine, omega-3, transition period

Adisseo Message:

Supplying methionine to dairy cows has been observed to improve milk fat production. Additionally, supplying dietary fatty acids is known to alter the fatty acid profile of milk. This abstract therefore was focused on the milk fatty acid composition of cows fed rumen-protected methionine (RP-Met) and calcium salts (CS) enriched in omega-3 fatty acids. Offering RP-Met and CS enriched in n3FA improved the fatty acid profile of milk. The n-3FA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are known to have beneficial effects on metabolism in both animals and humans. Thus, producing milk with higher EPA and DHA can have health benefits for the consumer.

Monday, June 26th. ADSA-Graduate Student Competition: Production – Poster (PhD)

Feeding rumen-protected methionine and calcium salts enriched in omega-3 fatty acids modify plasma and liver phosphatidylcholine and phosphatidylethanolamine concentrations of periparturient cows

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POSTER 1024M

The objective of this study was to investigate the effects of feeding rumen-protected (RP)-Met and calcium salts (CS) of fatty acids (FA) enriched without or with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA; i.e., n3FA) in periparturient cows. In a randomized complete block study design, 75 multiparous Holstein cows were assigned to 1 of 4 treatments (n = 18-19/diet): 1) Met deficient (-Met) with CS not enriched in n3FA (-n3FA; 0% n3FA; EnerGII; Virtus Nutrition, USA), 2) Met adequate (+Met; Smartamine M; Adisseo Inc., France) with -n3FA, 3) -Met with CS enriched in n3FA (+n3FA; 4% n3FA; EnerG-3; Virtus Nutrition), or 4) +Met with +n3FA from wk -3 prior to expected calving through wk 4 of lactation. Cows were fed corn silage-based total mixed rations, pre- and postpartum, which were formulated to provide Met at ≤0.96 or ≥1.13 g per Mcal metabolizable energy (ME) for -Met and +Met, respectively. CS were fed at 1.5% ration dry matter pre- and postpartum. Plasma and liver samples collected on d 21 relative to parturition were analyzed for phosphatidylcholine (PC), phosphatidylethanolamine (PE), and lysophosphatidylcholine (LPC) by LC-MS. Data were analyzed using PROC MIXED of SASv9.4. Planned contrasts included: 1) effect of Met (-Met vs. +Met), 2) effect of n3FA (-n3FA vs. +n3FA), and 3) effect of co-supplementation (+Met/+n3FA vs. +Met/-n3FA and -Met/+n3FA). Liver LPC-20:5 and -22:6 concentrations were greater in +n3FA, relative to -n3FA ($P < 0.01$). We observed greater plasma LPC-22:6 in +Met/+n3FA, relative to +Met/-n3FA and -Met/+n3FA ($P < 0.001$). Liver PC-38:6 concentrations were greatest in +n3FA, relative to -n3FA ($P < 0.001$), which were negatively correlated with liver triglyceride concentrations (r = -0.25; $P = 0.04$). Liver PE-38:6 concentrations were greater in +Met/+n3FA, relative to +Met/-n3FA and -Met/+n3FA ($P < 0.001$). A lower liver PC-38:6:PE-38:6 was observed in +Met/+n3FA, relative to +Met/-n3FA and -Met/+n3FA ($P < 0.001$).

Feeding adequate methionine and omega-3 FA in the transition period modified plasma and liver PC and PE profiles.

Keywords: methionine, omega-3, transition

Adisseo Message:

Feeding methionine during the peripartum period has been observed to have beneficial effects on liver metabolism. Omega-3 fatty acids (n3FA) have also been documented to alter metabolism. However, no work has investigated the effects of supplying both n3FA and methionine during the peripartum period.

Even though there are not known requirements for polyunsaturated fatty acids by dairy cows, feeding them in conjunction with rumen-protected (RP) methionine resulted in changes in phosphatidylcholine and phosphatidylethanolamine in plasma and liver, reinforcing the beneficial effects reported previously on DMI, milk production and composition, and liver health.

The results from this trial underscore the importance of feeding eicosapentaenoic acid and docosahexaenoic acid along with a RP-source of methionine to high producing dairy cows during the peripartum period.

Monday June 26th: Production, Management, and the Environment 1

Rumen-protected methionine supplementation during the transition period under heat stress: impact on cow-calf performance

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ORAL 2182

Dairy cows exposed to heat stress (**HS**) during the dry period produce less milk and milk protein in the next lactation and give birth to smaller calves. Rumen-protected methionine (**RPM**) has been shown to modulate protein, energy, and placenta metabolism, suggesting RPM may be a nutritional intervention to mitigate adverse HS effects. We examined the effects of RPM supplementation to transition cows under HS induced by electric heat blankets (**EHB**) on cow-calf performance. Fifty-three Holstein cows were housed in a tie-stall barn 6 weeks before expected calving and fed a control diet (**CN**, 2.2% Met of MP) or a CN diet with Smartamine®M (**MT**, 2.6% Met of MP, Adisseo Inc., France). Four weeks pre-calving all MT and half CN cows received an EHB. The other half of the CN cows were left at thermoneutrality (**TN**) resulting in: CNTN, CNHS, MTHS (n=17-19). Respiration rate (**RR**) and skin temperature (**ST**) were measured thrice weekly. Post-calving body weights (**BW**) and BCS were recorded weekly. Daily milk yield was recorded, and components were analyzed every third day. Calf birth weight and stature were measured, and apparent efficiency of absorption (**AEA**) of immunoglobulins was calculated. Data were analyzed using the MIXED procedure of SAS with 2 orthogonal contrasts: CNTN vs. the average of CNHS and MTHS (**C1**) and CNHS vs. MTHS (**C2**). The use of EHB increased RR (C1: 39.0 vs. 51.9 ± 1.4bpm) and ST (C1: 31.2 vs. 34.4 ± 0.1°C), relative to TN (P<0.0001). Post-calving BW, BCS, and milk yield were not impacted by the EHB (P≥0.36), however, protein % was reduced by EHB (C1: 3.3 vs. 3.2 ± 0.04%, P=0.07) but SNF was not (C1: 9.2 vs. 9.2 ± 0.06%, P=0.6). Protein % (C2: 3.1 vs. 3.3 ± 0.05%) and SNF (C2: 8.9 vs. 9.4 ± 0.07%) were significantly lower in CNHS, relative to MTHS (P≤0.005). Calf birthweight (C1: 42.7 vs. 39.5 ± 0.96kg) and AEA (C1: 53.8 vs. 38.9 ± 5.2%) were reduced by HS (P≤0.02), and wither heights tended to be shorter in CNHS (C1: 78.6 vs. 77.4 ± 0.6cm; P=0.13; C2: 76.5 vs. 78.2 ± 0.7cm; P=0.09),

compared to MTHS heifers. Overall, RPM supplementation to transition cows reverts the negative impact of HS on milk protein and calf wither heights.

Adisseo Message:

As climate change occurs, heat stress will continue to cause losses for the livestock industry. Supplemental methionine during the peripartum period has been increasing on commercial farms due to the beneficial effect of methionine on milk production, health, and metabolism, as well as on the developing fetus. This study aimed to further understand the beneficial effects of methionine during the peripartum period by examining the effects of rumen-protected methionine supplementation during exposure to heat stress in late gestation and early lactation. The results underscore the negative effects of heat stress on milk protein production and birth weight of calves. However, consistent with previous work using late-lactation cows (Pate et al., 2020), methionine supplementation helped to mitigate the negative effects of heat stress on milk protein.

Monday June 26th: ADSA-USD Competition: Original Research Poster Presentations

Molecular and gene expression changes in liver tissue from mid-lactation dairy cows supplemented with methionine during a subclinical mastitis challenge.

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POSTER1034M

The objectives were to assess molecular changes in the liver of cows supplemented with methionine during solely a subclinical mastitis challenge (SMC). Thirty-two multiparous Holstein cows (145±51 DIM) were enrolled in a randomized complete block design and assigned to either a basal diet (CON; n=16) or a basal diet supplemented with rumen-protected methionine (SM; n=16, Smartamine M 0.09% DM). The dietary treatment was administered at -21 days relative to a SMC, and data were collected from 0 to 3 days. At 0 d relative to SMC, the mammary gland rear right quarter was infused with 500,000 cfu of Streptococcus uberis (0140J). Blood samples were collected during SMC to assess inflammation, oxidative stress, and liver function biomarkers. Liver biopsies were taken -10 and 1 d relative to SMC. Genes related to methionine and glutathione metabolism, inflammatory response, and oxidative stress were analyzed via qPCR and western blots. Data were analyzed using the PROC MIXED procedure of SAS with TRT and TRT*TIME interaction as an effect. Significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$. There was no TRT*TIME interaction for any of the evaluated genes. A trend ($P = 0.07$) for greater milk yield (+0.9 kg) was observed in SM cows than CON. Methionine metabolism genes *MAT2A* and *PEMT* were upregulated ($P = 0.02$) in SM cows compared with CON. *BHMT* was downregulated ($P = 0.03$) in SM cows compared with CON. Compared to CON, a trend ($P = 0.09$) was observed for upregulation of *MAT1A* in SM cows. Immune cell signaling gene *MYD88* was downregulated ($P = 0.01$) in SM cows compared with CON. A trend ($P = 0.06$) in haptoglobin (*HP*), a positive acute-phase response, was observed, where *HP* was downregulated in SM cows compared with CON. *A transcription factor involved in antioxidant regulation, NRF2, was upregulated (P = 0.01) in* SM compared with CON. A trend

(*P* = 0.09) was observed in glutathione synthetase (*GSS*), which directs the final step in glutathione biosynthesis, where *GSS* was upregulated in SM cows compared with CON. The lower reduction in milk yield in SM cows during the SMC could be associated with upregulation of antioxidant-related genes and downregulation of inflammatory-related genes.

Keywords: Methionine, Inflammation, Oxidative Stress

Adisseo Message:

Feeding methionine during the peripartum period is becoming a common practice in commercial herds. Cows fed Smartamine M during this period have shown increased DMI and milk production, have experienced lower incidence of post-calving metabolic disorders and have had higher plasma antioxidant status. This trial was designed to determine the metabolic response of cows fed Smartamine M infected by infusing *Streptococcus uberis* to the rear right quarter of the mammary gland. Methionine is not an antibacterial drug, but rather it is a required nutrient that has functional properties. The results of this work demonstrate how methionine affects metabolic pathways that impact antioxidant-related genes, and therefore provides meaningful insights that help to explain the better health and higher milk production of cows after calving.

Tuesday June 27th: Ruminant Nutrition 4 - Calves and Heifers

Long-term impacts of in-utero heat stress on heifer feed efficiency and enteric gas emissions

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ORAL 2478

As global climate changes, producers may face the consequences of increased heat stress events during the dry period on performance and efficiency of the cow and her unborn calf as evidenced by previous research. Studies regarding the feed efficiency of lactating cows are readily available, but data regarding the feed efficiency of growing heifers are limited. Our objective was to evaluate the long-term effects of in-utero heat stress on subsequent heifer performance and greenhouse gas emissions. A total of 38 heifers had been subjected to heat stress (HT; n = 17) or artificial cooling (CL; n = 21) in-utero (last 56 d of gestation) and were enrolled in a 63-day study at 18-20 months old. Heifers were blocked by weight and randomly assigned to 3 pens with Calan gates and access to a GreenFeed machine (GF; C-Lock, SD) for 8 ± 1d to measure CH_4 and CO_2 gas fluxes. Body weights (BW) were measured on days -2, -1, 0 and 61, 62, 63 and used to calculate average daily gain (ADG). Hip height (HH), hip width (HW), and chest girth (CG) were recorded on days 0 and 63. All heifers were fed the same TMR consisting of 46.6% oatlage, 44.6% grass/alfalfa haylage, 7.7% corn silage, 0.3% urea, and 0.8% mineral (DM basis). Statistical analyses were performed using R version 4.1.1 (R Core Team, 2021) with packages lme4, and emmeans. Residual feed intake (RFI) was calculated by subtracting predicted DMI from observed DMI. The HT and CL heifers did not differ in initial (lsmean ± se; 551 ± 8.6 kg, 543 ± 7.8 kg; $P = 0.47$, respectively) or final BW (615 ± 10.1 kg, 607 ± 9.1 kg; $P = 0.57$, respectively). There were no treatment effects ($P > 0.1$) on DMI (HT: 12.0 ± 0.46 kg, CL: 12.0 ± 0.46 kg DM) or ADG (HT: 1.00 ± 0.04 kg/d, CL: 1.02 ± 0.03 kg/d). RFI was similar between HT (-0.009 ± 0.1) and CL (0.007 ± 0.1, $P = 0.90$). Methane production, CO2 production, and visits to the GF did not differ between treatment ($P > 0.5$). Despite previously reported reductions in growth and feed intake of in-utero heat stress heifers during the pre-weaning phase, it does not seem to have long-term effects on growth, feed efficiency, or methane emissions later in life.

Adisseo Message:

Exposure to heat stress during the peripartal period has negative consequences not only for the cow, but also the unborn calf. As climate change continues to be an issue, it is important to understand how in-utero exposure to heat stress can impact heifers long-term, During the pre-weaning period, calves exposed to in-utero heat stress have been reported to have reduced performance, as well as lower milk yields when they reach lactation. Despite those previous outcomes, the results of this study suggest that in-utero heat stress during late gestation does not seem to affect heifer performance during the growing phase.

Tuesday June 27th: Ruminant Nutrition: Protein and Amino Acids 2

Effects of rumen-protected methionine on the plasma amino acid profile of F1 Holstein × Gyr cows grazing intensively managed Mombaça grass.

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POSTER 1526T

The objective of this study was to evaluate the effect of rumen-protected Met (RPM; Smartamine M; Adisseo Inc., France) supplementation on plasma amino acid (AA) profile of lactating grazing cows. Twenty-four multiparous F1 Holstein x Gyr cows (31.0 kg/d, 100 DIM) were used in a randomized block design for 9 weeks to evaluate the supplementation of a control diet with RPM to adjust Lys:Met ratio from 3.1 to 2.8 (CNCPS). The supplementation of RPM varied from 10 to 15 g/d, depending on the production of the cow. Cows grazed as a group and were allotted to a new paddock of Mombaça grass every day using sward height as the target entry criteria. Cows were milked twice daily, and individually fed a concentrate mixture three times a day, after each milking and at 11:00 am. Blood samples were collected from a coccygeal vessel into heparinized test tubes at 11:00 am, before concentrate was fed, on the last day of weeks 3, 6 and 9. The samples centrifuged and plasma was stored at – 20°C until analysis. Plasma AA concentration was analyzed by isotopic dilution, liquid chromatography, and electrospray ionization mass spectrometry after derivatization. The model included treatment, week and week x treatment interaction as fixed effects, in addition to block as random effect. Least square means were compared using Tukey's test. The supplementation of RPM did not increase plasma Met concentration (35.7 vs. 31.4 µmol/L, P = 0.22). Plasma concentration of essential AA tended to increase for the RPM treatment (878.4 vs 811.4 µmol/L, P = 0.06), while concentration of non-essential AA tended to decrease for RPM (1229.0 vs 1307.2 µmol/L, P = 0.08). More specifically, RPM reduced plasma concentration of Glu (46.6 vs. 50.9 umol/L, P = 0.05), Gly (308.3 vs. 350.0 umol/L, P = 0.03) and tended to decrease plasma concentration of Gln (194.8 vs. 215.0 umol/L, P = 0.07). Milk protein yield was higher (894 vs 835 g/d, P = 0.01) for RPM. These results suggested that Met was a deficient AA in the control diet and that and increase in plasma Met is not necessary for a positive production effect of RPM supplementation.

Keywords: Protein, tropical pasture

Adisseo Message:

There are a limited number of research trials feeding RP-Met in grazing systems with tropical grasses in South America during the summer season. The leaves contain high levels of crude protein, up to 25%, depending on growth stage and nitrogen supply. To our knowledge, this is the first report of plasma amino acids concentration of lactating cows fed Smartamine M on pasture systems.

The results of this work underscore the importance of the methionine's metabolic elasticity. The points to rescue from the results from this trial are:

1) The reported plasma concentration of methionine is relatively high on Girolando cows in pasture fed cows.
2) Cows fed Smartamine had numerically higher plasma methionine concentration, however its concentration was not statistically higher than cows not supplemented with Smartamine M.
3) Despite the cows fed the basal diet had relatively high plasma methionine concentrations, when supplemented with Smartamine M, responded with higher milk protein production.

Monday June 26th: Physiology and Endocrinology 1

Systemic transcriptomic analysis in lactating cows with elevated peripheral serotonin

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POSTER 1141M

The objective of this study was to investigate the systemic transcriptional response to elevated peripheral serotonin in lactating cows. In a cross-over design, multiparous Holstein cows (187±34 DIM, n=8) were intravenously infused with saline or the serotonin precursor 5-HTP (1mg/kg bodyweight) on days 1–3 and 8–10 of each period, for 1h/d, starting at 7am. Adipose tissue (WAT), liver, and mammary were biopsied 6 h after d10 infusion. Extracted RNA was sequenced, mapped to the cattle reference genome (ARS-UCD 1.2), and analyzed with DEseq2 for differentially expressed genes (DEG) using a combinatory cutoff of mean read-count ≥ 5, fold-change ≥ 2, and p-value < 0.01. 5-HTP downregulated 71 and upregulated 14 identified genes in WAT, 59 genes were downregulated and 95 upregulated in the liver, and 43 downregulated and 29 upregulated in mammary tissue. Expression patterns were analyzed by QIAGEN Ingenuity Pathway Analysis (IPA) with absolute Z-score ≥ 2 as cut-off. Based on IPA prediction, 5-HTP decreased activity of 54 upstream regulators of the DEG in WAT, including insulin, PPAR gamma, and FOXO-1; and increased activity of 17 regulators. In line, *synthesis and metabolism of triacylglycerol* and *uptake of monosaccharides* were predicted to be inhibited in response to 5-HTP. In the mammary glands, 5-HTP increased the activity of 90 upstream regulators and decreased activity 220 of them. In addition, IPA predicted that 5-HTP increased mammary lipids trafficking. In the liver, 90 upstream regulators had decreased activity, including insulin receptor, PKC, FOXO-1 and 2, and SREBF-1 and 2; and 61 had increased activity, including the cholesterol synthesis inhibitors INSIG-1 and 2. In line, the *superpathway of cholesterol biosynthesis* was predicted to be inhibited by 5-HTP in the liver. In conclusion, lactating cows with elevated peripheral serotonin developed a systemic response, altering the expression of genes in energy metabolic pathways, including reduced lipid synthesis in WAT, reduced cholesterol synthesis in the liver, and increased lipid trafficking in the mammary tissue. Together, these changes may function to shift energy partitioning towards milk synthesis.

Adisseo Message:

Among its many functions in the body, serotonin plays a role in energy metabolism. 5-hydroxytryptophan (5-HTP) is the precursor to serotonin and infusing 5-HTP has been documented to increase peripheral serotonin concentrations in lactating dairy cows. This study aimed to investigate the effects of serotonin on liver, mammary and adipose tissue metabolism using 5-HTP. In particular, changes across the three tissues indicated a systemic change in lipid metabolism, suggesting a shift in energy partitioning towards milk rather than storage in adipose tissue.

Monday June 26th: Ruminant Nutrition 2 | Protein and Amino Acids

Production responses of dairy cows receiving jugular infusion of methionine and lysine or leucine and isoleucine.

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ORAL PRESENTATION 2203: Shaw Center 212 10:45 AM

Previous studies showed that essential amino acids (EAA) affect *de novo* fatty acid (FA) synthesis, especially Lys, Met, Leu, and Ile. We aimed to assess production responses in cows supplemented with 2 groups of EAA. Twelve Holstein cows (117±29 DIM and 41±5.3 kg/d of milk production) were randomly assigned to Lys/Met or Ile/Leu groups and subsequently assigned to treatment sequences within 2 orthogonally replicated 4 x 3 Youden squares (6 repetitions per treatment). Treatments were with or without Lys (37.6 g/d) or Met (12.0 g/d), and with or without Ile (28.6 g/d) or Leu (41.0 g/d) arranged as 2 x 2 factorials. Treatments were administered through jugular infusion for 10 days. Cows were fed a basal diet (1.77 Mcal of NEL/kg and 9.55 % of MP). On the last day of each period, 2-[^{13}C]-acetate was infused for 24 h as a tracer for *de novo* FA synthesis. Data were analyzed at R software, using EAA as fixed effects and period, cow, and square as random effects. Tukey test was used for multiple comparisons ($P < 0.05$). Leucine or Ile infusion does not affect DMI (18.7 kg/d SEM=0.42). Leucine infusion reduced ($P = 0.04$ and $P = 0.05$) energy-corrected milk (2.6 kg/d, SEM=0.92) and milk protein production (40 g/d, SEM=0.03). Isoleucine infusion did not change milk production, but it reduced ($P = 0.01$) milk protein concentration (0.16 percentual units, SEM = 0.04) and increased ($P = 0.01$) 0.08 percentual units milk lactose (SEM=0.02). No effect on milk protein was observed from the combination of Ile+Leu. Milk fat concentration, milk fat production (g/d), and milk mixed FA (palmitic and palmitoleic) proportion tended to increase ($P = 0.06$, $P = 0.09$, and $P = 0.07$) for Ile+Leu infusion. On the other hand, Ile+Leu tended to reduce ($P = 0.06$) *de novo* milk FA proportion. Methionine infusion tend to decrease ($P = 0.08$) DMI (from 19.7 to 18.6 kg/d, SEM=0.42). However, infusion of Lys/Met did not affect milk production and composition responses, except by a tendency to decrease ($P = 0.07$) milk lactose concentration by infused Met. Isoleucine and Leu additively affected milk fat synthesis and independently affected ECM, milk protein, and milk lactose. Under the conditions of the study, Met and

Lys did not independently or additively affect milk production and composition responses in dairy cows.

Keywords: amino acids, essential amino acid, milk fat synthesis

Adisseo Message:

The response of feeding some amino acids to dairy cows has been studied for over 60 years. It is thoroughly documented that cows supplemented with methionine show an increase in milk protein production. More recently, it has been reported that supplementing diets with essential amino acids not only increases milk protein synthesis via the mechanistic target of rapamycin (mTOR) signaling pathway but also de novo fatty acid synthesis through mTOR and the transcription factor sterol-regulatory-element-binding protein 1 (SREBP1).

This trial was designed to evaluate the impact of some mTOR-signaling amino acids on de novo milk fatty acid production. Diets in the trial were formulated to meet the requirements at the predicted dry matter intake of the cows. However, the lower-than-expected dry matter intake, therefore, the lower-than expected intake of essential amino acids, may have prevented the cows from responding to the treatments.

Despite the results of this trial do not support the original hypothesis, there was a tendency for an increase in milk fat concentration and production from the cows supplemented with Ile + Leu highlighting the potential of the BCAA on the cows' ECM production outcome.