



## Research on Fat Nutrition - Conference Summary 2022

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## Key points from conference abstracts

### Oleic acid – 3 papers

- Data relating to the beneficial effects of oleic acid (C18:1) on improving the energy status of dairy cows by reducing lipolysis / enhancing lipogenesis.
- Also improved metabolic state through increased plasma insulin and reduced NEFA and BHB.
- Further data to support our approach of our Ca salt products being most appropriate during early lactation to support body condition.
- One paper reporting the beneficial effects of supplying oleic acid as a Ca salt rather than ruminal infusion, with improved digestibility of NDF and FA reported for the Ca salt form. This evidence provides additional support for our Ca salt brands in delivering oleic acid through the rumen for beneficial effects.

### Palmitic acid – 6 papers

- Some evidence of C16 influencing C18:2 oxidation to reduce inflammatory responses, but more data needed to determine if these have metabolic effects.
- Additional study reporting 1.8 kg/d increase in milk yield and increased milk fat by 0.26% by supplementation of C16 at 2% of DM.
- A meta-analysis study reports that C16 supplementation increased mixed FA (C16:0, C16:1) in milk but without decreasing de novo or preformed FA, and some increase in C18:1, potentially for milk fat fluidity purposes.
- Heifers offered C16 for three weeks from calving showed significant increases in milk fat in week two (+0.36%) and week three (+0.37%), but no effects recorded on DM intake or milk yield across the trial period.
- *In vitro* work showed that high rumen USFA load reduced fibre digestibility, acetate and total VFA concentrations, but addition of C16 was not able to overcome these negative effects. These data support the use of rumen-protected fats and minimising the USFA load in the rumen.

## Key points from conference abstracts

### Milk processing – 7 papers

- Some data looking at factors affecting FFA in milk but a lot of variation not explained, and FFA reduce the frothing properties of milk. Low milk fat is associated with better frothing, though one study indicated that higher fat milks frothed well as long as FFA was minimised.
- Inclusion of C16 in the diet was reported to have no effects on cheese quality attributes, and had no effect on raw milk FFA concentration, casein micelle size and fat globule diameter.
- A further study showed that cows supplemented with Ca salts of PFAD produced softer butter oil whereas a high-C16 supplement increased hardness.
- These are further data to support our discussions on the effect of different dietary FA on milk fatty acid profile and dairy product functionality.

### Fat digestibility – 8 papers

- Studies looking at the effects of lecithin phospholipids reported little effect on performance or digestibility parameters, indicating these are not an effective method of increasing FA digestibility in supplements.
- However, benefits in FA digestibility were reported with Tween 80 polysorbate infused into either the rumen or abomasum.





**Oleic acid**

## 1095 Oleic acid limits lipolysis and improves mitochondrial function in adipose tissue from periparturient dairy cows.

- U. Abou-Rjeileh\*<sup>1</sup> , D. Salcedo<sup>1</sup> , J. Parales<sup>1</sup> , C. Prom<sup>1</sup> , M. Chirivi<sup>1</sup> , N. J. O'Boyle<sup>2</sup> , J. Laguna<sup>1</sup> , A. L. Lock<sup>1</sup> , and G. A. Contreras<sup>1</sup> , <sup>1</sup> Michigan State University, East Lansing, MI, <sup>2</sup> University of Nottingham, Loughborough, United Kingdom.
- **Oleic acid (OA) has been shown to modulate lipid mobilization and enhance mitochondrial function.** In the liver, OA promotes lipid droplet formation by activating perilipin 5 (PLIN5) and peroxisome proliferator activated receptor  $\alpha$  (PPAR $\alpha$ ); however it is unknown if this mechanism occurs in adipose tissue (AT). We hypothesize that OA limits lipolysis and improves mitochondrial function in bovine AT. Multiparous Holstein cows (n = 12) were infused abomasally following parturition with ethanol (CON) or OA (60 g/d) for 15 d. Subcutaneous AT samples were obtained at  $11 \pm 3.6$ d before calving (PreP), and  $6 \pm 1$ d (PP1) and  $13 \pm 1.4$ d (PP2) after parturition. Adipocyte morphometry was performed on H&E-stained sections. Isoproterenol (ISO, 1 $\mu$ M) stimulated lipolysis and insulin (1 $\mu$ g/L) inhibition of ISO were determined using an in vitro explant culture by measuring glycerol release. PLIN5 and PPAR $\alpha$  expression were determined by capillary electrophoresis. NGS RNA sequencing was used to evaluate the transcriptomic profile of bioenergetics gene pathways. Statistical analyses were performed using a mixed effect model which included the random effect of cow, and the fixed effect of treatment, time, and their interactions. At PP2, compared with CON, OA reduced AT response to ISO and increased AT sensitivity to insulin (P < 0.01). Compared with CON, OA decreased the percentage of smaller adipocytes (< 0.001) hence limiting adipocyte size reduction. Compared with CON, OA tended to have higher PPAR $\alpha$  content at PP1 (P < 0.10), and increased PLIN5 protein expression at PP2 (P < 0.05). At PP2, OA increased PARP3 fragments per kilobase million mapped reads (FPKM) which mediates DNA strand break repair (P < 0.05) and tended to increase SOD2 FPKM which plays an antiapoptotic role against oxidative stress (P = 0.06). OA decreased SIRT3 FPKM at PP2 (P < 0.05) supporting a shift to lipogenesis. **Our results provide initial evidence that OA may limit lipolysis by enhancing lipogenesis through the activation of PPAR $\alpha$  through PLIN5.** RNA-seq results show that OA improves mitochondrial integrity reflecting a state of lipogenesis.

## 1096 Rumen vs. abomasal infusion of oleic acid as an approach to determine the potential for an oleic acid-enriched calcium-salt to affect digestibility and production of dairy cows.

A. M. Burch\*<sup>1</sup>, J. de Souza<sup>2</sup>, and A. L. Lock<sup>1</sup>, <sup>1</sup> Michigan State University, East Lansing, MI, <sup>2</sup> Perdue AgriBusiness, Salisbury, MD.

We determined the effects of a high oleic acid (OA; C18:1) Ca-Salt (23% C16:0 and 64% C18:1) alongside rumen and abomasal infusion of OA on digestibility and production responses of lactating dairy cows. Eight multiparous cows ( $46.2 \pm 5.96$  kg/d of milk;  $161 \pm 11$  DIM) were assigned to treatment sequences in a replicated 4x4 Latin square design with 18-d periods, consisting of 7-d of washout and 11-d of infusion. Treatments were: water infusions (CON), abomasal infusion of OA (ABO), ruminal infusion of OA (RUM), or rumen supplementation of a high OA Ca-salt (SALT). Treatments delivered 50 g/d of C18:1. Cows were fed the same diet that contained (%DM) 30% NDF, 16% CP, 30% starch, and 3.1% FA. The statistical model included the random effect of cow within square and the fixed effects of period, treatment, and their interaction. Pre-planned contrasts were: CON vs the average of the 3 C18:1 treatments (CON vs FAT), OA infusion in the abomasum vs rumen (ABO vs RUM) and OA ruminal infusion vs Ca-salt (RUM vs SALT). Results are presented in the following order: CON, ABO, RUM, and SALT. Treatment had no effect on DMI or NDF intake ( $P > 0.39$ ). FAT tended to decrease 16-carbon FA digestibility (62.2, 62.8, 55.5, 61.3;  $P = 0.09$ ) compared with CON. FAT increased 3.5% FCM (42.6, 44.8, 43.9, 43.3 kg/d;  $P = 0.03$ ), decreased milk fat content (3.56, 3.44, 3.45, 3.47%;  $P = 0.03$ ), and tended to increase milk protein yield (1.31, 1.37, 1.34, 1.33 kg/d;  $P = 0.07$ ) and content (3.01, 3.04, 3.03, 3.06%;  $P = 0.07$ ) compared with CON. ABO increased digestibility of NDF (48.4 vs 46.3,  $P = 0.04$ ), 16-carbon FA ( $P < 0.01$ ), 18-carbon FA (62.0 vs 56.2,  $P = 0.01$ ), and total FA (62.5 vs 56.4,  $P = 0.01$ ) compared with RUM. SALT increased digestibility of DM (67.0 vs 69.1,  $P < 0.01$ ), NDF (46.3 vs 49.0,  $P = 0.01$ ), 16-carbon FA ( $P < 0.01$ ), 18-carbon FA (56.2 vs 60.2;  $P = 0.04$ ), and total FA (56.4 vs 60.8;  $P = 0.03$ ) compared with RUM. In summary, abomasal infusion of OA and rumen supplementation of a high OA Ca-salt improved the digestibility of NDF and FA compared with ruminal infusion of OA in mid-lactation dairy cows.



## 2131M Abomasal infusion of oleic acid improves plasma hormones and metabolites in early lactation dairy cows.

J. M. dos Santos Neto\*, U. Abou-Rjeileh, J. Parales-Giron, C. M. Prom, G. A. Contreras, and A. L. Lock, Michigan State University, East Lansing, MI.

Our objective was to determine whether abomasal infusion of oleic acid (cis-9 C18:1) improves hormonal and metabolic responses in early lactation cows. Twelve rumen-cannulated multiparous cows were used in a randomized complete-block design and assigned to treatments from 1 to 15 DIM. Treatments were abomasal infusions at 6 h intervals of ethanol carrier only (CON) or 60 g/d oleic acid (OA). Cows were fed the same diet which contained (% DM) 32.5% NDF, 17.2% CP, 25.5% starch, and 1.96% fatty acids (FA). We evaluated production responses over 15 d, blood parameters at 3, 5, 7, 10, 12, and 14 d, digestibility once, and a glucose tolerance test (GTT) on d 14. For GTT, blood samples were taken -10, -1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 150, and 180 min relative to the dextrose infusion via a jugular catheter. The model included the random effect of block, cow within block and treatment, Julian date, and the fixed effects of treatment, time, and their interactions. We used repeated measures, except for digestibility. We did not observe interactions between treatment and time for any production variable ( $P \geq 0.16$ ). Infusing OA did not affect digestibility ( $P \geq 0.50$ ) or production ( $P \geq 0.21$ ). Compared with CON, OA increased plasma insulin (0.08 uIU/mL;  $P < 0.01$ ) and decreased plasma glucose (5 mg/dL;  $P = 0.01$ ), NEFA (0.07 mEq/L;  $P = 0.05$ ), and BHB (2.69 mg/dL;  $P = 0.04$ ). We observed an interaction between treatment and time for BHB ( $P = 0.01$ ), wherein OA reduced BHB at 5 ( $P < 0.01$ ) and 7 d ( $P = 0.03$ ) compared with CON. During the GTT, we observed interactions between treatment and time for both plasma insulin ( $P < 0.01$ ) and glucose ( $P = 0.04$ ). Compared with CON, OA increased insulin peak at 10 min (5.02 uIU/mL;  $P < 0.01$ ) and also increased it at 20 min (1.40 mg/dL,  $P = 0.03$ ) and tended to increase it at 30 min ( $P = 0.07$ ). Compared with CON, OA decreased glucose peak at 10 (17.5 mg/dL), 20 (12.3 mg/dL,  $P < 0.01$ ), 30 (5.75 mg/dL), and 40 min (5.96 mg/dL,  $P < 0.05$ ). In conclusion, abomasal infusion of oleic acid increased plasma insulin and decreased plasma glucose, NEFA, and BHB, suggesting an improvement in metabolic state of early lactation dairy cows.



**C16 – palmitic acid**



## 2072M Chromium and palmitic acid supplementations modulate oxidized linoleic acid metabolite biosynthesis in periparturient dairy cows.

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Periparturient lipolysis increases linoleic acid (C18:2 n-6) availability which is the substrate for oxidized linoleic acid metabolites (OXLAM). Among OXLAM, 9 and 12- hydroxy-octadecadienoic acids (HODE) are proinflammatory, whereas 9- and 13-oxo-octadecadienoic acids (oxoODE) and 13-HODE facilitate inflammation resolution and promote lipogenesis. Chromium supplementation improves systemic insulin sensitivity, possibly reducing lipolysis. Palmitic acid (PA) feeding increases nutrient availability for milk production. This study evaluated the effect of feeding Cr and PA on OXLAM production. Multiparous Holstein cows were selected at -21d before parturition. After calving, cows were randomly assigned to one of 4 diets containing: 1) no supplementation (CON, n = 4); Chromium propionate (Cr, at 0.45 ppm Cr/kg DM, n = 4); PA (1.5% DM, n = 4); or Cr+PA (n = 4) that were fed from 1 to 21 DIM. Plasma and subcutaneous AT (SCAT) biopsies were collected at -13 ± 5.1 d prepartum (PreP) and 14.4 ± 1.9 d (PP1), 21 ± 1.9 d (PP2) postpartum. Targeted lipidomic analysis was performed using HPLCMS/MS. The statistical model included the fixed effect of diet, time, and treatment, the random effect of the block, cow, and cow nested in diet and block. Results are presented in the following sequence: CON, Cr, PA, Cr+PA. In plasma, 9-, 12-, 13-HODE and 9- and 13-oxoODE increased at PP1 and PP2 compared with PreP (P < 0.001). Postpartum, Cr and PA reduced 9-HODE compared with CON and Cr+PA (95.2; 75.39; 66.6; 98.2 ± 9 nM; P < 0.05). Similar results were observed for 13-HODE (85.8 ± 7.3; 71.3 ± 7; 62.37 ± 7; 85.0 ± 7 nM; P < 0.05). PA and Cr+PA increased 13-oxoODE compared with Cr but not to CON (2.7; 1.9; 2.9; 3.2 ± 0.3 nM; P < 0.05). 9-oxoODE was lower in PA compared with other treatments (17.7; 17.9; 13.3; 21.6 ± 2.3; P < 0.05). In AT, 9-, 13-HODE increased postpartum (P < 0.05). Postcalving, there were no effects of diet or diet\*time on OXLAM content in AT. Cr and PA appear to reduce HODE and potentiate the production of oxoODE. The impact of these changes in OXLAM biosynthesis on metabolic functions warrants further investigation.

## 2362W Effect of dietary palmitic acid supplementation and milking frequency on milk production and composition in early lactation dairy cows.

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This study was conducted to evaluate if combined effect of feeding palmitic acid (PA) and increasing milking frequency (MF) may benefit milk responses of early lactation dairy cows. Eight multiparous Holstein cows averaging  $45 \pm 14$  DIM were used in a replicated  $4 \times 4$  Latin square design with a  $2 \times 2$  factorial arrangement of treatments to study the effects of dietary PA (0 or 2% on a DM basis), MF (twice- or thrice-daily, at regular intervals), and their interaction on milk production and composition. Treatment periods were 21 d in length, with the last 5 d used for data and sample collection. Dry matter intake was not affected by treatment ( $28.2 \pm 0.8$  kg/d;  $P \geq 0.32$ ), and no interaction between PA and MF was observed on any measured parameters ( $P \geq 0.16$ ). Regardless of MF, supplementing cows with PA increased milk (48.0 vs. 46.2 kg/d;  $P < 0.01$ ), energy-corrected milk (ECM; 47.0 vs. 43.9 kg/d;  $P < 0.01$ ), and 4% fat-corrected milk (FCM; 47.9 vs. 44.5 kg/d;  $P < 0.01$ ) yields along with ECM:feed efficiency (1.67 vs. 1.56;  $P < 0.01$ ). Compared with no supplementation, feeding dietary PA increased milk fat concentration (4.03 vs. 3.77%;  $P < 0.01$ ) and yield (1.92 vs. 1.73 kg/d;  $P < 0.01$ ), as well as protein yield (1.54 vs. 1.47 kg/d;  $P < 0.01$ ), whereas milk protein concentration was not affected ( $3.21 \pm 0.07\%$ ;  $P = 0.34$ ). Thrice-daily MF increased milk (48.6 vs. 45.6 kg/d;  $P < 0.01$ ), ECM (46.6 vs. 44.3 kg/d;  $P < 0.01$ ), and FCM (47.4 vs. 45.0 kg/d;  $P < 0.01$ ) yields along with ECM:feed efficiency (1.65 vs. 1.58;  $P = 0.01$ ). Increasing MF decreased milk fat concentration (3.84 vs. 3.96%;  $P = 0.05$ ) but increased milk fat yield (1.86 vs. 1.79 kg/d;  $P = 0.01$ ). Whereas milk protein concentration was not affected ( $3.21 \pm 0.07\%$ ;  $P = 0.24$ ), protein yield was increased (1.55 vs. 1.46 kg/d;  $P < 0.01$ ) with MF. Milk free fatty acid concentration was similar among treatments ( $0.70 \pm 0.07$  mEq/100g of milk fat;  $P \geq 0.70$ ). This study demonstrated that both PA supplementation and thrice-daily MF increased milk and fat yields, but the absence of interaction reveals that their effects were additive.

## 1091 Meta-analysis examining the effect of palmitic acid supplementation on molar changes in de novo and preformed milk fatty acids in dairy cows.

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We performed a meta-analysis to evaluate the effect of palmitic acid (C16:0) supplementation on changes in milk FA yield in moles per day (mol/d). Our analysis included 1,215 individual observations from 16 studies conducted in early to late lactation cows. Treatments were control diets (CON) with no supplemental FA (n = 557) and diets supplemented at 1.5% DM with C16:0-enriched supplements or FA blends containing  $\geq 80\%$  C16:0 (PA; n = 658). Diets (% DM) included (mean  $\pm$  SD)  $29.8 \pm 2.1$  NDF,  $27.2 \pm 2.2$  starch, and  $16.9 \pm 0.51$  CP. Production across treatments was  $28.5 \pm 3.4$  kg/d DMI,  $45.5 \pm 4.5$  kg/d milk,  $1.70 \pm 0.22$  kg/d milk fat, and  $1.45 \pm 0.13$  kg/d milk protein. The statistical model included the random effect of study, cow within study, and period or day of treatment within study. Sources of milk FA were classified as de novo (16 carbons). Compared with CON, PA had no effect on de novo yield (P = 0.22) but increased C4:0 yield (P < 0.01) by 4.4% and decreased yields of C8:0, C10:0, C12:0, and C14:0 by 5.0%, 9.6%, 11.0%, and 6.1%, respectively (P < 0.01). Compared with CON, PA increased mixed yield (P < 0.01) by 16.5% and increased C16:0 and C16:1 yields (P < 0.01) by 17.3% and 8.4%, respectively. PA had no effect on preformed yield (P = 0.69) but decreased C18:0 yield by 2.5% and increased C18:1 yield by 3.2% (P < 0.01) compared with CON. Overall, the relative molar yield of FA sources for CON were 34.3% de novo, 33.0% mixed, and 32.6% preformed compared with 32.4% de novo, 36.7% mixed, and 30.9% preformed for PA. In conclusion, our results indicate that C16:0 supplementation increases the inclusion of mixed FA into milk fat without decreasing the yields of de novo or performed FA. However, C16:0 supplementation does shift de novo synthesis toward greater C4:0 yield and lower yields of longer-chain de novo FA and shifts preformed FA yield toward greater C18:1 rather than C18:0. This could be due to positional distribution of specific FA in triglycerides for maintenance of milk fat fluidity.



## 1092 Effect of palmitic acid supplementation on production responses of primiparous dairy cows during early lactation.

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Palmitic acid (C16:0) supplementation has been shown to increase energy partitioning toward milk production, improving the yields of milk fat, 3.5% FCM, and ECM in multiparous cows at different stages of lactation, including early lactation. In the current study we evaluated the effect of C16:0 supplementation on DMI, the yields of milk and milk components, and BW and BCS of primiparous dairy cows during early lactation. Twenty-eight first-lactation Holstein cows ( $2,359 \pm 130$  GTPI) were used in a complete randomized block design and assigned to either a control diet containing no supplemental fat (CON), or a diet supplemented with a C16:0-enriched supplement at 1.5% diet DM (PA) from calving to 23 DIM. The diets contained (% DM) 26.2% forage NDF, 25.1% starch, and 17.9% CP. The C16:0-enriched supplement (91% C16:0) replaced soyhulls in CON. The study ran from August 2019 to March 2020, when it had to be stopped due to COVID-19 pandemic restrictions. The statistical model included the random effects of block, cow within block and treatment, and Julian date, and the fixed effects of treatment, time, and their interactions. When the interaction between treatment and time was significant, mean comparisons were made within week of lactation. Treatment did not affect DMI, milk yield, milk protein content and yield, or BW and BCS change ( $P > 0.34$ ). PA tended to increase the yields of milk fat ( $P = 0.07$ ) and 3.5% FCM ( $P = 0.09$ ). Treatment interacted with time; in the second week of lactation, PA increased milk fat content (4.40, 4.76 %;  $P < 0.01$ ) and the yields of milk fat (1.23, 1.42 kg/d;  $P < 0.05$ ), 3.5% FCM (32.2, 36.0 kg/d;  $P < 0.01$ ), and ECM (31.7, 34.9 kg/d;  $P < 0.05$ ), and tended to increase DMI ( $P = 0.09$ ). In the third week of lactation, PA increased milk fat content (4.07, 4.44%;  $P < 0.05$ ) and tended to increase milk fat yield ( $P = 0.06$ ). These data provide evidence for beneficial effects of palmitic acid supplementation in early lactation primiparous cows. However, the fewer number of experimental units than initially intended did reduce the statistical power and ability to detect treatment differences.

## 2286W Chromium and palmitic acid supplementation modulate adipose tissue insulin sensitivity in periparturient dairy cows.

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Periparturient dairy cows exhibit intense lipolysis driven by reduced dry matter intake, enhanced energy needs, and the loss of adipose tissue (AT) insulin sensitivity (IS). Extended periods of low IS induce lipolysis dysregulation, leading to increased disease and poor lactation performance. **Chromium (Cr) supplementation improves systemic IS**, while palmitic acid (PA) feeding increases energy availability for milk production. However, the effect of feeding Cr alone or with PA on IS in AT is unknown. Our goal was to determine the effect of chromium (Cr) and palmitic acid (PA) supplementation on AT IS. Multiparous Holstein cows were selected at -21d before parturition. After calving, cows were randomly assigned to one of 4 diets that were fed from 1 to 21 DIM. Control, no supplementation (CON, n = 8); Cr (Cr-propionate at 0.45 ppm Cr/kg DM, n = 8); PA (1.5% DM, n = 8); or Cr+PA, (n = 8). Plasma (for NEFA quantification) and subcutaneous AT (SCAT) explants were collected at  $-13 \pm 5.1$ d prepartum (PreP) and  $14.4 \pm 1.9$ d (PP1) and  $21 \pm 1.9$ d (PP2) postpartum. SCAT were incubated in the presence of the lipolytic agent isoproterenol (ISO = 1  $\mu$ M, BASAL = 0  $\mu$ M) for 3 h. The antilipolytic effect of insulin (1 $\mu$ g/L) was evaluated during ISO stimulation (IN+ISO). Lipolysis was quantified by glycerol release in the media (nmol glycerol/mg AT). The statistical model included the random effect of the block, cow within block and diet, Julian date, and the fixed effect of diet, time, treatment, and their interactions. BASAL lipolysis at PP1 and PP2 was higher in Cr-fed cows ( $0.45 \pm 0.06$ ;  $P < 0.01$ ), compared with CON, PA, and Cr+PA ( $0.32$ ,  $0.19$ , and  $0.22 \pm 0.06$ ). In Cr cows, ISO induced higher lipolysis ( $2.49 \pm 0.18$   $P < 0.01$ ) compared with Cr+PA ( $1.54 \pm 0.18$ ), but not different from CON and PA ( $1.94$  and  $2.03 \pm 0.18$ ). IN+ISO reduced lipolysis ( $1.7 \pm 0.22$ ) in Cr cows compared with ISO ( $2.49 \pm 0.18$ ). In contrast, IN+ISO did not affect ISO lipolysis in CON, PA, and Cr+PA ( $P < 0.01$ ). At PP1 and PP2, plasma NEFA was lower in Cr cows ( $0.48 \pm 0.05$ ), compared with CON and Cr+PA ( $0.57$ ,  $0.56 \pm 0.05$   $P < 0.05$ ), but not different from PA ( $0.50 \pm 0.05$ ) Our results demonstrate that supplementation with Cr enhances IS. The higher basal and ISO lipolysis rates suggest higher lipid accumulation in AT by improved IS. Further studies should determine the effects of Cr on AT lipogenesis and adipogenesis.

## 2524V Palmitic acid supply and rumen unsaturated fatty acid load on rumen fermentation in a continuous culture system.

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Oversupply of rumen unsaturated fatty acids (RUFAL) can disrupt the cell membrane function and permeability of rumen microorganisms. Nonrumen bacteria incorporate palmitic acid as a mechanism to reduce membrane permeability. We hypothesized that rumen bacteria use a similar mechanism, thereafter, providing saturated fatty acids in the diet could support bacterial metabolism under RUFAL. The objective of this study was to evaluate the effects of dietary palmitic acid associated with RUFAL level on rumen fermentation. The study was conducted as a 2 × 2 factorial treatment arrangement in a replicated 4 × 4 Latin square using continuous culture fermenters (n = 8). Treatments were a) a control diet without supplemental fatty acids (FA), b) the control diet plus 1.5% palmitic acid, c) the control diet with palmitic acid plus Low RUFAL (2.0% of diet DM), d) the control diet with palmitic acid plus High RUFAL (4.0% of diet DM). Soybean oil and soyhulls were used to create variation in RUFAL levels. The control diet (40 g DM/day) was a 50:50 orchardgrass hay:concentrate mixture. Daily fermenter effluent was collected over 24-h post-feeding and a 30% subsample was pooled by fermenter within period. Data were analyzed using a mixed model including the fixed effect of FA, RUFAL, and its interaction, and the random effects of period and fermenter. The differences were declared at  $P \leq 0.05$  and tendencies at  $P \leq 0.10$ . No interaction between FA and RUFAL was observed. Treatments with High RUFAL decreased fiber digestibility, total VFA concentration, and acetate concentration ( $P \leq 0.04$ ). Palmitic acid increased fiber digestibility and total VFA concentration ( $P \leq 0.03$ ). Butyrate concentration was not affected by treatment. Our preliminary results indicate that RUFAL negatively impacts rumen fermentation and palmitic acid is not able to overcome its negative effect.





## **Dietary fatty acids and milk properties**

## 1073 Identifying on-farm factors associated with the level of free fatty acids in bulk tank milk.

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Elevated concentrations of free fatty acids (FFA) in bulk tank milk are a recent concern in the dairy industry. FFA result from triglyceride hydrolysis and milk with > 1.2 mmol FFA/100g of milk fat is associated with undesirable characteristics, such as off-flavor, rancidity, reduced frothing ability, and inhibited cheese coagulation. Previous research indicates that elevated FFA are multifactorial, and this study aimed to identify the major contributing factors at the farm level. We hypothesized that automated milking systems (AMS), fat additives in the lactating ration, and a narrow pipeline diameter are associated with higher concentrations of FFA. An observational cross-sectional study was conducted to identify on-farm factors associated with elevated FFA in bulk tank milk. A total of 300 Canadian dairy farms in Ontario (240) and British Columbia (60) were visited once to complete a survey, assess milking systems, and gather ration data. Bulk tank FFA values for each farm were obtained from the provinces' milk marketing boards and a monthly FFA average around the farm visit date was used as the outcome variable. Univariable and multivariable linear regression analyses were used to identify explanatory variables associated with bulk tank FFA. Of the 300 study farms, 70 were tie-stall, 110 were freestall with parlors, and 120 were freestall with AMS. The mean bulk tank FFA was 0.83 mmol FFA/100g of fat (SD = 0.39, range = 0.26 to 3.67) and 9.0% (27) of herds had elevated FFA. Contrary to our hypothesis, milking pipeline diameter was not associated with FFA concentration. In the final multivariable linear regression model, tie-stall milking systems ( $\beta = 0.32$ ,  $P < 0.001$ ), increased milking frequency ( $\beta = 0.30$  per milking,  $P < 0.001$ ), and the use of fat supplements in the lactating ration ( $\beta = 0.09$ ,  $P = 0.04$ ) were associated with higher FFA concentration. Pre-cooling milk was associated with lower FFA ( $\beta = -0.16$ ,  $P = 0.016$ ). However, these variables explained only 23% of the variability in FFA in bulk milk, which suggests that more factors affecting FFA still need to be identified.

## 1203 Fat content and processing of shelf stable milk—Which factors do influence the frothing properties?

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Coffee specialties as latte macchiato and cappuccino increase in popularity over the past decade. Low-fat milk is associated with good frothing properties; despite free fatty acids impairing the frothing behavior of milk. An increased fat content elevates the risk for enzymatic breakdown of fat, since more substrate is available for the enzymatic reaction. Thus milk with higher fat content is more likely to possess bad frothing properties. The impairment of milk froths by free fatty acids was already shown to be critical for raw and pasteurized milk, though industry also faces problems with batches of shelf stable milk forming bad foams. Shelf stable milk is defined as ultra-high-temperature treated milk with a shelf life of 4 mo or longer. In this study, milk was preheated for 128 s at 90°C and ultra-high-temperature treated for 4 s at 141°C. It was homogenized with a pressure of 25/5 MPa to obtain a stable emulsion. How does the cow breed and the processing of the milk influence the free fatty acid content and the frothing behavior in the final product? Lipolysis was induced homogenizing (10/2 MPa) fresh raw milk at a temperature of 20°C of 2 different cow breeds. Kinetics were assessed based on the extent of mechanical stress induced by application of different homogenization pressures (10/2 MPa, 15/3 MPa, 20/4 MPa, 25/5 MPa), resulting in lipolysis. The development of the free fatty acid content during milk processing was examined with a spectroscopic (MIR) milk analyzer. Milk originated lipase was inactivated by pasteurization, as soon as the desired content of free fatty acids was obtained. Subsequently, UHT treatment and homogenization were performed. Milk was frothed under reproducible conditions inducing a steam-air mixture into the sample and total sample volume was determined over a period of 15 min. A free fatty acid content of >2.0 mEquiv / 100 g fat was found to be critical regarding frothing behavior of shelf stable milk with a fat content of 3.5 % (wt/wt). The influence of milk's fat content on frothing behavior was investigated, producing milk with 3.5, 4, 6, 7 and 8 % (wt/wt) fat. Further, the influence of septic and aseptic homogenization with the aforementioned fat contents on the frothing behavior was assessed. Emulsion stability was examined performing microscopy and particle size analysis via laser scattering. Septic homogenization was shown to not significantly ( $\alpha = 0.05$ ) influence emulsion stability negatively for samples with a fat content of up to 6 % (wt/wt). Samples of higher fat content showed fat globule agglomeration. Emulsions of septically homogenized samples of all regarded fat contents remained stable. For fat contents of up to 8 % (wt/wt) it was shown, that frothing capacity is not negatively influenced by fat, as long as free fatty acid level was not elevated.



## 2007M Effect of dietary palmitic acid supplementation and milking frequency on cheese-making properties of milk.

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Palmitic acid supplementation and greater milking frequency can increase fat yield and milk production in dairy cows, but the impacts of those practices on milk processing are still unclear. The objective of this study was to evaluate the effects of dietary palmitic acid, thrice-daily milking frequency, and their interaction on cheese yield and composition. Eight multiparous early lactation Holstein cows ( $45 \pm 14$  DIM) were used in a replicated  $4 \times 4$  Latin square design including 4 periods of 21 d, with a  $2 \times 2$  factorial arrangement of treatments. Cows received a diet with or without palmitic acid (0 or 2% on a DM basis) and were milked twice- or thrice-daily, at regular intervals. At each period, milk from consecutive milkings of d 18 and 19 was collected from each cow and pooled by treatment in 250-L refrigerated bulk tanks and transferred to the Université Laval for cheese manufacture at pilot scale (10-L vats). No difference between treatments was observed for raw milk free fatty acid concentration ( $0.61 \pm 0.18$  mEq/100 g of milk fat), casein micelle size ( $156.2 \pm 7.7$  nm), and fat globules diameter (d<sub>3,2</sub>  $1.52 \pm 0.88$   $\mu$ m; d<sub>4,3</sub>  $3.80 \pm 0.33$   $\mu$ m). Before Cheddar cheese manufacture, milk was standardized to a constant casein:fat ratio ( $0.779 \pm 0.009$ ), and casein ( $3.27 \pm 0.03\%$ ) and fat ( $4.20 \pm 0.02\%$ ) contents. Cheese moisture in nonfat substances ( $52.2 \pm 1.1\%$ ) and fat in dry matter content ( $48.5 \pm 0.4\%$ ) were similar among treatments. Similarly, treatments had no effect on moisture-adjusted yield ( $12.5 \pm 0.1\%$ ) and fat recovery ( $93.5 \pm 1.0\%$ ). A tendency was observed for a greater protein recovery in cheese from thrice-daily milking ( $83.6\%$  vs.  $82.2\%$ ;  $P = 0.10$ ). Results suggest that with a standardized cheese-making procedure, palmitic acid supplementation and milking frequency do not affect cheesemilk performance. Further results should include the effects of palmitic acid supplementation and milking frequency on cheese composition and properties during ripening.

## 1543V Effect of lipid source supplementation on production responses and butter oil texture in dairy cows.

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- Our objective was to evaluate the effect of feeding different lipid sources varying in the fatty acid (FA) profile on production responses and butter oil texture (hardness and cohesiveness) on lactating dairy cows. Twelve mid-lactating cows were fed a basal ration (DM basis 54.1% roughage and 45.9% concentrate) were used in a replicated 6x3 incomplete truncated Latin square with 3-wk periods. Treatments were a control diet (CON) that did not receive supplementary FA, or the inclusion of 1% (DM basis) of FA supplementation of a saturated FA (SFA; C16:0+C18:0; Energy Booster 100; Milk Specialties Global), a Ca salts of a palm FA distillate (PFAD; C16:0+C18:1; Perdue Ca Salts, Purdue AgriBusiness LLC) or a palmitic acid-enriched source (PA; C16:0; Spectrum Fusion, Purdue AgriBusiness LLC). Milk yield and composition and butter oil texture were evaluated in the last 4 d of each period. For butter oil texture, anhydrous butter oil was obtained by centrifugation of the milk cream with Tween 80; and determined by constant-speed penetrometry. Data were analyzed as a T-protected test using a mixed model, considering the fixed effect of treatment and the random effect of cow and period. Addition of PA increased ( $P < 0.05$ ; Table 1) dry matter intake (DMI), fat and protein yields, milk energy output (MEO), and butter oil hardness compared with CON. Cows supplemented with SFA had similar DMI than PA, CON, and PFAD; and greater protein yield than CON. Supplementation with PFAD decreases butter oil hardness. There was no effect of FA supplementation on butter oil cohesiveness ( $P = 0.41$ ). In conclusion, the FA profile of supplemental fat impacted production responses and butter oil properties.

## 1543V Effect of lipid source supplementation on production responses and butter oil texture in dairy cows.

**Table 1 (Abstract 1543V).** Effect of lipid source supplementation on DMI, MY and composition, MEO, and butter oil texture

Item	CON	SFA	PFAD	PA	SEM	<i>P</i> -value
DMI, kg/d	26.23 <sup>a</sup>	27.08 <sup>ab</sup>	26.70 <sup>a</sup>	27.43 <sup>b</sup>	0.78	<0.01
MY, kg/d	33.50	34.68	34.19	35.93	2.047	0.12
Fat, kg/d	1.40 <sup>a</sup>	1.48 <sup>ab</sup>	1.49 <sup>ab</sup>	1.55 <sup>b</sup>	0.101	0.01
Protein, kg/d	1.08 <sup>a</sup>	1.14 <sup>bc</sup>	1.10 <sup>ab</sup>	1.18 <sup>c</sup>	0.056	<0.01
MEO, Mcal/d	24.6 <sup>a</sup>	26.0 <sup>ab</sup>	25.7 <sup>a</sup>	27.1 <sup>b</sup>	1.53	0.01
Hardness, g	25,906 <sup>a</sup>	26,561 <sup>ac</sup>	24,126 <sup>b</sup>	28,098 <sup>c</sup>	2,127	<0.01
Cohesiveness, g	-16,67.4	-1,672.9	-1,745.6	-1,537.8	167.2	0.41



## 1542V Increasing amount of a palmitic acid in the diet increases milk fat yield, milk energy output, and butter oil texture in dairy cows.

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The objective of this experiment was to evaluate the effect dietary increased level of inclusion of a source of palmitic acid-enriched supplement (PAES) on production responses and butter oil texture (hardness and cohesiveness) on lactating dairy cows. Twelve mid-lactating cows were fed a control ration (DM basis 21.3% alfalfa haylage, 29% corn silage, 9.6% cotton seed, 3.8% rye silage, and 36.3% concentrate) were used in a replicated 6x3 incomplete truncated Latin square with 3-wk periods. Treatments were increasing levels of a PAES (0, 0.5, 1 or 2% of diet DM; Spectrum Fusion, Purdue AgriBusiness LLC, Salisbury, MD). Milk yield and composition and butter oil texture were evaluated in the last 4 d of each period. For butter oil texture, anhydrous butter oil was obtained by centrifugation of the milk cream with Tween 80; and the texture was determined by constant-speed penetrometry. Data were analyzed as polynomial contrast (linear, L; Quadratic, Q, and cubic, C) using a mixed model considering the fixed effect of treatment and the random effect of cow and period. Increasing PAES affected (Table 1) dry matter intake (DMI, P Q < 0.01) fat yield, milk energy output (MEO) and butter oil hardness. However, increase in PAES did not affect (P ≥ 0.23) butter oil cohesiveness. In conclusion milk yield and composition and butter oil hardness can be affected by the amount of PAES in lactating dairy cows.

**1542V Increasing amount of a palmitic acid in the diet increases milk fat yield, milk energy output, and butter oil texture in dairy cows.**

**Table 1 (Abstract 1542V).** Effect of increasing amount of a palmitic acid enriched supplement on DMI, MY and composition, MEO, and butter oil hardness and cohesiveness

Item	PAES, %				SEM	P-value		
	0	0.5	1	2		L	Q	C
DMI, kg/d	26.2	27.9	27.5	26.8	0.78	0.74	<0.01	0.02
MY, kg/d	33.5	35.1	35.9	34.7	2.05	0.18	0.02	0.88
Fat, kg/d	1.40	1.44	1.55	1.56	0.099	<0.01	0.34	0.21
Protein, kg/d	1.08	1.13	1.18	1.12	0.055	0.12	<0.01	0.49
Lactose, kg/d	1.55	1.64	1.69	1.61	0.105	0.16	<0.01	0.79
MEO, Mcal/d	24.6	25.6	27.1	26.5	1.50	<0.01	0.04	0.27
Hardness, g	25,906	27,505	28,098	28,078	2,148.9	<0.01	0.11	0.75
Cohesiveness, g	-13,091	-12,877	-13,285	-13,614	615.33	0.23	0.74	0.58



**Fat digestibility**



## 2264T Abomasal infusion of increasing doses of lecithin does not affect nutrient digestibility or production responses in postpeak dairy cows.

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In ruminants, lecithin is secreted into the abomasum aiding emulsification and micelle formation which are necessary for fatty acid (FA) absorption. To evaluate if supplemental lecithin could improve FA absorption, we determined the effects of abomasal infusions of increasing doses of lecithin on digestibility and production of dairy cows. Eight multiparous cows ( $76 \pm 9$  DIM;  $51.2 \pm 6.15$  kg/d of milk) were assigned to treatment sequences in a replicated 4x4 Latin square design with 18-d periods (7-d of washout and 11-d of infusion). **Treatments were abomasal infusions of water (0G), 15 g/d lecithin (15G), 30 g/d lecithin (30G), or 45 g/d lecithin (45G).** The product was de-oiled soybean lecithin containing 94% DM phosphatidylcholine and 52% DM total FA (18.5% C16:0, 3.5% C18:0, 7.3% C18:1, 62.5% C18:2, and 6.6% C18:3). Cows were fed the same diet of (%DM) 29% NDF, 17% CP, 27% starch, and 3.62% FA. The statistical model included the random effect of cow within square and the fixed effects of period, treatment, and their interaction. Pre-planned linear, quadratic, and cubic contrasts of increasing lecithin levels were tested. Results are presented in the following order: 0G, 15G, 30G, and 45G. Increasing lecithin dose increased 18-carbon FA intake (linear; 767, 778, 809, 800 g/d;  $P = 0.04$ ) and tended to increase 16-carbon (linear; 253, 256, 266, 262 g/d;  $P = 0.09$ ) and total FA intake (linear; 1,074, 1,088, 1,132, 1,117 g/d;  $P = 0.06$ ). There were no effects on DMI and NDF intake ( $P > 0.14$ ), or digestibility of DM, NDF, 16-carbon FA, or total FA ( $P > 0.32$ ). **Increasing lecithin dose affected 18-carbon FA digestibility (quadratic; 63.5, 60.4, 61.5, 62.5%;  $P = 0.09$ ).** **There were no effects of infusing lecithin on production responses ( $P > 0.25$ ).** In summary, **abomasal infusions of up to 45 g/d of de-oiled lecithin did not impact digestibility or production responses.** Lack of response could be due to the phospholipid profile of the lecithin, negative feedback from lecithin infusions reducing lecithin secretion by the cow, and a possible requirement for the conversion of lecithin to lysolecithin.



## 2130M Effect of soy phospholipids in a saturated fatty acid supplement on digestibility and production responses of mid-lactation dairy cows.

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We determined the effects of increasing the amount of soy phospholipids (SPL) incorporated into a saturated fatty acid (FA) supplement on digestibility and production responses of dairy cows. Sixteen multiparous ( $182 \pm 55$  DIM;  $56.3 \pm 8.62$  kg/d) and 8 primiparous ( $148 \pm 65$  DIM;  $49.7 \pm 3.25$  kg/d) cows were assigned to treatment sequences in a replicated 4x4 Latin square design with 14-d periods. Treatments were SPL inclusions of: 1) 0% SPL (CON), 2) 1.5% SPL (L-SPL), 3) 3.0% SPL (M-SPL), and 4) 4.5% SPL (H-SPL) which were incorporated into a saturated FA supplement containing 34% C16:0 and 45% C18:0. Treatment diets contained (%DM) 30% NDF, 16.5% CP, 27.8% starch, and 3.5% FA. The statistical model included the random effect of cow within square and the fixed effects of period, treatment, and their interaction. Pre-planned contrasts were the linear, cubic, and quadratic effects of increasing SPL. Results are presented in the following order: CON, L-SPL, M-SPL, and H-SPL. There was a linear effect on DMI (28.6, 28.9, 29.2, 29.1 kg/d;  $P = 0.05$ ), a linear tendency for NDF intake (8.62, 8.80, 8.86, 8.79 kg/d;  $P = 0.06$ ), a cubic effect for 16-carbon FA intake (240, 238, 248, 247 g/d;  $P = 0.02$ ), and a linear effect for 18-carbon FA intake (696, 696, 714, 717 g/d;  $P < 0.01$ ) and total FA intake (965, 969, 987, 995 g/d;  $P < 0.01$ ). There was a quadratic effect on digestibility of DM (66.9, 65.1, 65.6, 66.2%;  $P < 0.01$ ) and NDF (48.8, 46.5, 46.8, 47.7%;  $P = 0.01$ ), and a cubic effect on digestibility of 16-carbon FA (70.5, 68.9, 72.7, 72.4%;  $P < 0.01$ ), 18-carbon FA (64.8, 60.2, 66.8, 65.2%;  $P = 0.01$ ), and total FA (65.9, 62.4, 67.8, 66.6%;  $P = 0.02$ ). There was a cubic effect on milk fat content (3.96, 3.91, 4.01, 3.93%;  $P < 0.01$ ) and a linear tendency for milk protein yield (1.35, 1.36, 1.36, 1.37 kg/d;  $P = 0.09$ ). There was no effect of treatment on other milk production responses, BW, or BCS (all  $P > 0.11$ ). In summary, increasing SPL in a saturated FA supplement had minimal impact on digestibility and milk production. We did observe an increase in FA digestibility which was primarily driven by the supplement containing 3.0% SPL.

## 2357W Effect of feeding whole cottonseed on nutrient digestion and fecal flow of intact seeds in dairy cows.

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Whole cottonseed is fed unprocessed and intact seeds are sometimes seen in manure resulting in questions about digestibility. Four primiparous and 8 multiparous Holstein cows were arranged in a 4x4 Latin square design with 21 d periods to investigate total-tract digestibility of WCS. Treatments were 0, 3.4, 6.8, and 9.9% WCS substituted for cottonseed hulls and soybean meal to balance NDF and crude protein. Total-tract (TT) digestibility was evaluated using indigestible NDF as a flow marker. Additionally, intact seed in feces were quantified by wet sieving with a 4.75mm sieve and analyzed for fatty acid concentration. Data were analyzed using the mixed procedure in JMP Pro 16. Cow and period were random effects and treatments was a fixed effect. Preplanned contrasts tested the linear and quadratic effect of increasing cottonseed level. Increasing WCS decreased TT DM by 1.4, 3.2, 4.4 and NDF digestibility by 4.8, 7.3, 10.4 percentage units respectively ( $P < 0.001$ ), but increased TT OM digestibility in both primiparous and multiparous cows ( $P < 0.001$ ). Total FA digestibility was not changed by WCS level, but 18C FA tended to linearly decrease with increasing cottonseed ( $P = 0.09$ ). In addition, WCS increased 16C FA digestibility linearly ( $P < 0.04$ ), but with a quadratic response in multiparous cows. There was a linear increase in the total flow of intact WCS (242 to 762 seeds/d;  $P < 0.001$ ), but there was no change in intact WCS as a percentage of seeds consumed ( $P = 0.94$ ). An average 2.3% of WCS consumed escaped digestion and appeared intact in the feces but was higher in multiparous cows than primiparous cows (4.3% vs 1.2%). The intact seeds averaged 20.4% FA while the WCS fed was 16.7% FA. Also, there was a linear increase in the amount of total FA lost in seeds (3.38, 6.55, and 8.88 g of FA/d) and multiparous cows voided more cottonseed FA in feces than primiparous. However, when expressed as a percent of cottonseed FA consumed, there was no difference between parities. Overall, feeding more cottonseed improved OM digestibility and reduced the amount of fat lost in feces, although NDF digestion was reduced.

## 2358W A meta-regression evaluating the effect of increasing dietary whole cottonseed on nutrient digestibility and production responses of lactating dairy cows.

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We performed a meta-regression to evaluate each 1-percentage-unit increase of whole cottonseed (WCS) in diet DM on nutrient digestibility and production responses of lactating dairy cows. The data set comprised 48 peer-reviewed publications containing at least one control diet (CON; no WCS inclusion) and a diet with WCS included at  $\leq 17\%$  diet DM. Publications comprised 25 change-over and 25 continuous studies. CON diets (% DM) contained (mean  $\pm$  SD)  $34.6 \pm 6.42$  NDF,  $17.4 \pm 1.55$  CP, and  $3.44 \pm 1.52$  FA. WCS diets (% DM) contained  $35.6 \pm 7.49$  NDF,  $17.6 \pm 1.78$  CP, and  $4.48 \pm 1.29$  FA. Cows averaged  $29.5 \pm 6.23$  kg of milk/d. We calculated the difference between WCS means minus CON means, resulting in one observation per WCS-CON pair. The meta-regression was performed using PROC MIXED of SAS and included the fixed effects of the slope due to WCS in diet, the random effect of study, and residual error. There were no interactions between treatment and experimental design for any production variable ( $P \geq 0.25$ ). Each 1-percentage-unit increase of WCS in diet DM had no effects on CP digestibility ( $P = 0.43$ ); decreased the digestibilities of DM (0.07 percentage units,  $P = 0.04$ ) and NDF (0.02 percentage units,  $P = 0.03$ ); and increased total fat digestibility (0.43 percentage units,  $P = 0.02$ ). Each 1-percentage-unit increase of WCS in diet DM had no effect on DMI ( $P = 0.71$ ), milk yield ( $P = 0.83$ ), BW ( $P = 0.16$ ), or BCS ( $P = 0.65$ ); and increased the yields of milk fat (0.003 kg/d,  $P < 0.01$ ), milk protein (0.002 kg/d,  $P = 0.05$ ), and ECM (0.06 kg/d,  $P = 0.01$ ). Each 1-percentage-unit increase of WCS in diet DM decreased the yield of de novo milk FA (1.57 g/d,  $P = 0.05$ ), increased the yield of mixed milk FA (1.31 g/d,  $P < 0.01$ ), and tended to increase the yield of preformed milk FA ( $P = 0.08$ ). In conclusion, each 1-percentage-unit increase of WCS in diet DM decreased DM digestibility, NDF digestibility, and de novo milk FA yield; and increased total fat digestibility, the yields of milk fat, milk protein, ECM, and mixed and preformed milk FA. Given the low average milk production in our data set, future research should determine the effect of increasing dietary WCS in high-producing cows.

## **1087 The form, more than the fatty acids profile of fat supplements, influences digestibility but not necessarily the production performance of dairy cows.**

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The form of fat supplement, degree of saturation, and the fatty acids (FA) profile influence the cows' productive response. The objective was to examine the effects of fat supplements differing in form or FA profile on nutrient digestibility and cows' performance. Forty-two dairy cows were assigned into 3 groups and fed for 13 wks rations contained (on DM basis): 1) CS45:35 – 2.4% of calcium salts of fatty acids (CSFA) comprised of 45% palmitic acid (PA) and 35% oleic acid (OA); 2) CS80:10 – 2.4% of CSFA comprised of 80% PA and 10% OA; 3) FF80:10 – 2.0% of free FA comprised of 80% PA and 10% OA. Rumen and fecal samples were taken for VFA and digestibility measurements, respectively. Production data were analyzed with PROC MIXED, and rumen and digestibility data with GLM models of SAS. Milk yields tended to be higher in FF80:10 than in CS80:10 cows ( $P = 0.07$ ). Fat percentage was highest in the FF80:10 (4.02%), intermediate in the CS80:10 (3.89%), and lowest in the CS45:35 cows (3.75%;  $P = 0.001$ ). The 4% FCM yields were highest in the FF80:10 cows and ECM yields were higher in the FF80:10 than in the CS80:10 cows. DMI did not differ among groups, and the calculated EB was lower in the FF80:10 cows ( $P = 0.0001$ ). Efficiency calculations for FCM and ECM were higher in the FF80:10 cows. The rumen acetate/propionate was lowest in FF80:10 ( $P = 0.0005$ ), and the total VFA was lowest in CS80:10 cows ( $P = 0.004$ ). Digestibility of DM and OM were higher in the CS80:10 than in FF80:10 cows ( $P = 0.03$ ), protein digestibility was lowest in the FF80:10 group ( $P = 0.02$ ), and NDF and ADF digestibilities were highest in CS80:10 cows ( $P = 0.0002$ ). Crude fat and 16-carbon FA digestibilities were lower in the FF80:10 ( $P = 0.05$ ), and that of 18-carbon FAs was lower in the FF80:10 than in the CS80:10 cows ( $P = 0.008$ ). In conclusion, the form, more than the FA profile of the fat supplements influences digestibility. However, it appears that energy partitioning toward production was higher in the FF80:10, although the digestibility was lower in this group than in cows fed both CSFA products.



## 2360W Altering the ratio of palmitic and stearic acids in supplemental fatty acid blends impacts digestibility responses of mid-lactation dairy cows.

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We determined the effects of altering the ratio of palmitic (C16:0; PA) and stearic (C18:0; SA) acids in supplemental fatty acid (FA) blends on digestibility responses of mid-lactation dairy cows. Twenty-four multiparous Holstein cows ( $109 \pm 23$  DIM;  $47.1 \pm 5.83$  kg/d of milk) were randomly assigned to treatment sequences in a replicated 4x4 Latin square design with 21-d periods. Treatments were a non-FA supplemented control diet (CON), and 3 diets incorporating 1.5% DM FA supplement blends, replacing soyhulls in the CON diet, containing 30% PA and 50% SA (L-PA), 50% PA and 30% SA (M-PA), and 80% PA and 10% SA (H-PA). FA blends were balanced to contain 10% oleic acid. Diets were formulated to contain (% DM) 31.0% NDF, 27.0% starch, and 16.9% CP. The statistical model included the random effect of cow within square and the fixed effects of period, treatment, and their interaction. Pre-planned contrasts included CON vs. overall effect of FA supplementation (FAT) and the linear and quadratic effects of increasing PA in the FA blends. Results are presented in the following order: CON, L-PA, M-PA, and H-PA. Compared with CON, FAT had no effect on DMI ( $P = 0.44$ ), decreased NDF intake (10.1, 9.67, 9.86, 9.88 kg/d;  $P < 0.01$ ), did not affect digestibility of DM ( $P = 0.32$ ) or NDF ( $P = 0.12$ ), but decreased the digestibility of 16-carbon (62.7, 58.3, 51.6, 48.1%;  $P < 0.01$ ), 18-carbon (66.8, 55.4, 58.0, 63.8%;  $P < 0.01$ ), and total FA (65.0, 56.2, 55.5, 56.5%;  $P < 0.01$ ). Increasing PA increased DMI (linear; 29.9, 29.4, 29.8, 30.0 kg/d;  $P = 0.04$ ) and NDF intake (linear;  $P = 0.04$ ). Increasing PA tended to decrease digestibility of NDF (linear;  $P = 0.06$ ) and decreased the digestibility of 16-carbon FA (linear;  $P < 0.01$ ) and increased the digestibility of 18-carbon FA (linear;  $P < 0.01$ ). In summary, feeding FA supplements containing PA and SA decreased NDF intake and decreased FA digestibility compared with a non-FA supplemented diet. Increasing the level of PA in the FA blend increased DMI, decreased NDF intake and 16-carbon digestibility but increased 18-carbon digestibility.

## 1090 Rumen and abomasal infusion of an exogenous emulsifier improves nutrient digestibility of lactating dairy cows.

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We evaluated the effects of infusing an exogenous emulsifier either into the rumen or abomasum on DMI and nutrient digestibility of lactating dairy cows. Nine rumen-cannulated multiparous cows ( $170 \pm 13.6$  DIM;  $43.6 \pm 5.20$  kg of milk) were assigned in  $3 \times 3$  Latin squares with 18 d periods consisting of 7 d of washout and 11 d of infusion with sampling on the last 4 d. Treatments were infusions of: 1) water carrier only into the rumen and abomasum (CON); 2) 30 g/d polysorbate-C18:1 (Tween80; T80) infused into the rumen (RUM); and 3) 30 g/d T80 infused into the abomasum (ABO). Cows were fed the same diet which contained (%DM) 32.2% NDF, 16.1% CP, 26.5% starch and 3.41% fatty acids (FA; 1.96% FA supplement containing 28% C16:0 and 55% C18:0). The statistical model included the random effect of cow within square and the fixed effects of treatment, period, square, and their interactions. We evaluated 2 orthogonal contrasts: 1) the overall effect of T80 infusions (CON vs. the average of ABO and RUM) and 2) RUM vs. ABO. Overall, T80 had no effect on DMI ( $P = 0.16$ ), decreased 16-carbon FA intake (4.50 g/d;  $P = 0.04$ ), and increased the digestibility of NDF (2.85 percentage units;  $P = 0.05$ ), total FA (4.65 percentage units), 16-carbon FA (3.25 percentage units), and 18-carbon FA (4.60 percentage units;  $P < 0.01$ ). Infusing T80 did not affect 16-carbon absorption ( $P = 0.20$ ) and tended to increase 18-carbon ( $P = 0.07$ ) and total FA ( $P = 0.09$ ) absorption. Compared with RUM, ABO had no effect on DMI ( $P = 0.16$ ) but decreased the intake of total FA (28 g/d;  $P = 0.02$ ), 16-carbon FA (7 g/d;  $P = 0.01$ ), and 18-carbon FA (19.0 g/d;  $P = 0.03$ ). Compared with RUM, ABO had no effect on the digestibility of NDF ( $P = 0.88$ ) or 16-carbon FA digestibility ( $P = 0.13$ ) but tended to increase total FA and 18-carbon FA ( $P = 0.07$ ) digestibility with no effect on the absorption of total FA, 16-carbon FA, or 18-carbon FA ( $P > 0.60$ ). In conclusion, infusion of polysorbate-C18:1 into both the rumen and abomasum increased total FA, 16-carbon FA, and 18-carbon FA digestibility. Responses were marginally better for the abomasum compared with rumen infusion.

## 2515V Effects of saturated fatty acids with lysophospholipids on production in lactating dairy cows.

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Palmitic (16:0) and stearic (18:0) acids have been widely used as fatty acid (FA) supplements for lactating dairy cows. Previous studies showed evidence that 16:0 FA is superior to 18:0 FA on increasing milk and fat yields, which occurs, at least in part, due to greater FA digestibility with 16:0 FA versus 18:0 FA. Lysophospholipids (LPL) are emulsifiers that may have a benefit on FA digestibility and thus increase the production benefit of these FA. We examined the effects of saturated fat sources (mainly 16:0 or 18:0) with or without LPL supplementation on DMI, milk yield, and milk components. Forty mid-lactation cows were used in a randomized block design and assigned to 1 of 4 diets: C16, FA (80% 16:0, 5% 18:0, 10% 18:1) at 1.5% of dietary DM; C18, FA (50% 18:0; 28% 16:0, 8% 18:1) at 1.5% of dietary DM; C16L, C16 with 0.05% LPL (dietary DM; hydrolyzed soy lecithin); C18L, C18 with 0.05% LPL (dietary DM). The experiment consisted of 10-d covariate followed by 6-wk data collection. Data were analyzed using the MIXED procedure (block and phase, random effects; FA, LPL, week, and their interactions, fixed effects). Dry matter intake, milk yield, and BW change did not differ among treatments. Milk fat content (4.30% vs. 3.88%;  $P = 0.02$ ) and milk fat yield (1.61 vs. 1.39 kg/d;  $P < 0.01$ ) were greater for C16 compared with C18, leading to greater ECM (42.5 vs. 39.6 kg/d;  $P < 0.01$ ) and ECM per DMI (1.58 vs. 1.46 kg/kg;  $P < 0.01$ ) for C16 versus C18. Milk NEL yield was also greater (29.3 vs. 27.3 Mcal/d;  $P < 0.01$ ) for C16 versus C18. Milk protein content and yield were not affected by treatments. Milk lactose concentration was greater for C18 versus C16, but lactose yield did not differ. We did not observe effects of LPL or interaction of FA by LPL on any production variables. **In conclusion, feeding C16 increased milk fat, leading to increased ECM compared with C18. No effect of LPL or interaction of FA by LPL on production in the current study was not in line with results from studies in the literature,** suggesting that more studies are needed to evaluate LPL with various dietary and nutritional composition in addition to its dosage level.



**Omega-3**



# 1011 Effects of increasing dietary omega-3 fatty acid concentrations on dairy cattle milk fat composition and reproduction.

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The use of fat in the diet of dairy cows has been questioned because of potential dry matter intake (DMI) and milk fat depression. However, evidence has suggested adding extruded flaxseed with high levels of omega-3 fatty acids to the diet at 5 to 12% on a dry matter basis (DM) could have positive impacts on milk fatty acid profiles and reproductive performance. Flaxseed-fed cows increased milk  $\alpha$ -linolenic acid (ALA) content 3.5 to 6.6 times compared with control cows. Milk eicosatetraenoic acid (EPA) levels increased 2-fold when fed extruded flaxseed at 4% of dietary DM. Alpha linolenic acid and EPA are omega-3 fatty acids that reduced the risk of chronic health issues in humans. Feeding extruded flaxseed reduced saturated fatty acid (SFA) levels in milk by up to 12.4%. Reduction of SFA has been shown to have human health benefits.

When fed extruded flaxseed at 4 to 5% of dietary DM, cows averaged 17 fewer days from first insemination to conception. Another study showed that cows fed whole flaxseed had no cases of embryo mortality, while cows fed micronized soybean showed 8% embryo mortality. The mechanism of these reproductive benefits is not fully understood, but evidence has suggested that increased omega-3 fatty acid intake could lower prostaglandin production and had a protective effect on the embryo. Several studies have looked at the effect of feeding extruded flaxseed on DMI with varying results. Some studies showed decreased DMI while others showed no effect or slightly increased DMI. Studies showed a variable effect on milk yield with either no effect or slightly increased (2.7%) milk yield when cows were fed extruded flaxseed at 4% of dietary DM. Milk fat percentage was reduced from 3.6% to 3.4% in flaxseed-fed cows compared with control cows. In summary, cows fed extruded flaxseed improved milk fatty acid profile and reproductive performance while having little effect on DMI and milk yield with a slight decreased in milk fat percentage.

## 1244 Effects of feeding rumen-protected methionine and calcium salts enriched in omega-3 fatty acids on lactation in periparturient dairy cows.

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The objective of this study was to investigate the effects of feeding rumen-protected (RP)-methionine (Met) and calcium salts (CS) of fatty acids (FA) enriched without or with C20:5 and C22:6 (i.e., n3FA) on milk production in periparturient cows. In a study with a randomized complete block design, 79 multiparous Holstein cows were assigned to 1 of 4 treatments (n = 19–20/diet): 1) Met unsupplemented (-Met) with CS palm oil not enriched in n3FA (-n3FA; 0% n3FA; EnerGII; Virtus Nutrition, USA), 2) Met supplemented (+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 -4 before 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  $\leq 0.96$  or  $\geq 1.13$  g /Mcal metabolizable energy for -Met and +Met, respectively. CS were fed at 1.5% FA (% ration dry matter). Pre and post-calving data were analyzed using PROC MIXED of SASv9.4. Pre-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). Although prepartum dry matter intake (DMI) was not modified by diet, +Met and +n3FA cows had greater postpartum DMI, relative to -Met and -n3FA, respectively ( $P < 0.05$ ). Yields of energy-corrected milk (58.5 vs. 55.3 kg/d; ECM) and fat-corrected milk (FCM), milk protein % and yields were greater in +Met, relative to -Met ( $P < 0.05$ ); milk fat yield and milk lactose % tended to be greater in +Met ( $P \leq 0.08$ ). Yields of ECM (58.1 vs. 55.7 kg/d), and milk fat, protein, and lactose yields, and lactose % were greater in +n3FA, relative to -n3FA ( $P < 0.05$ ); FCM yields tended to be greater in +n3FA. Milk protein % and fold-change increase in ECM (wk 1 to 4) were greater ( $P \leq 0.02$ ), and milk fat % tended to be greater ( $P = 0.10$ ), in +Met/+n3FA, relative to +Met/-n3FA and -Met/+n3FA. **In conclusion, feeding transition cows RP-Met and CS enriched in n3FA enhanced ECM yields and milk composition.**



## **Milk fat synthesis**

## 1089 Interaction between trans-10,cis-12 CLA and acetate supplementation on milk fat production and milk fatty acids.

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Acetate supplementation increases milk fat synthesis under normal dietary conditions. It is not known if acetate can recover milk fat synthesis when milk fat depression (MFD) is induced with trans-10,cis-12 conjugated linoleic acid (CLA). The objective of this study was to characterize the interaction between trans-10,cis-12 CLA and acetate supplementation on milk fat synthesis and milk fatty acid (FA) profile. Ten ruminally cannulated cows were arranged in a 4 × 4 Latin square with a 2 × 2 factorial treatment design with 7-d periods and 14-d washout periods. Treatment factors were CLA (10 g/d infused to the abomasum) or sodium acetate providing 600 g/d of acetate as a continuous ruminal infusion. Milk samples were collected at each milking during the last 2 d of each period and all variables were averaged across the period for analysis. Data were analyzed with a mixed model that included the fixed effect of acetate, CLA, and their 2-way interaction and the random effect of cow and period. Acetate supplementation decreased milk yield by 2 kg/d (P = 0.02), regardless of CLA supplementation. As expected, CLA supplementation decreased milk fat percentage by 40% (P < 0.001) and milk fat yield by 655 g/d (P < 0.001). Acetate increased milk fat percentage by 9% (P = 0.01) and there was no effect of acetate on milk fat yield (P = 0.34). There was no interaction between factors in any milk production variable. Acetate supplementation increased the concentration of 16 C FA by 7% (P < 0.001) and tended to decrease the concentration of FA >16 C (P = 0.08). Supplementation of CLA decreased the concentration of FA < 0.001 for both, respectively, and increased the concentration of FA >16 C by 20% (P < 0.001). There was no interaction between factors in any milk FA category. In conclusion, acetate supplementation did not recover milk fat synthesis when MFD was induced with trans-10, cis-12 CLA. Acetate supplementation did stimulate mammary lipogenesis regardless of occurrence of CLA-induced MFD.



## 1093 Effect of 2-hydroxy-4-(methylthio) butanoate blended in a fatty acid supplement on lactating dairy cow performance when feeding diets with increased risk for diet-induced milk fat depression.

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2-Hydroxy-4-(methylthio)butanoate (HMTBa) is a rumen available methionine analog which has been shown to reduce the risk of biohydrogenation induced milk fat depression as a rumen modifier. Blending HMTBa with a fat supplement may impact its rumen availability and digestibility of the supplement. Our objective was to investigate the effect of HMTBa blended with a FA supplement on milk fat depression and fatty acid digestibility. Fifteen multiparous Holstein cows were randomly assigned treatments in a 3 × 3 Latin square with 21 d periods. Each period consisted of 2 dietary phases. During the first 18 d, a moderate risk diet was fed (28.5% NDF, 0.75% soy oil) and during the final 3 d a high-risk diet was fed (28.0% NDF, 1.5% soy oil). The fat supplement was included at 1.5% DM in all diets. Treatments were fat supplements that contained 0% (CON), 3.7% (LOW), and 7.3% (HIGH) HMTBa in the FA prill targeting 0, 12.5 and 25 g/d of HMTBa. Digestibility was measured using iNDF as an internal marker. Data were analyzed using the MIXED procedure of SAS. The model included the random effects of cow and period and the fixed effects of treatment, phase, and their interaction. There was a treatment by phase interaction (P = 0.02) on milk yield, where yield decreased during the high-risk phase for CON cows and increased for LOW cows (45.7 vs 47.6 kg/d). The high-risk diet did not decrease milk fat as expected, however milk protein increased (2.94% vs 2.96%, P = 0.02) and lactose decreased (4.85% vs 4.83%, P = 0.05) between phases. There were no effects of treatment on milk fatty acid profile, though there were phase effects. Both de novo and mixed source FA proportions decreased, while total 18 carbon FA, trans-10 C18:1 and trans-11 C18:1 increased in the high-risk phase. Total-tract DM digestibility was lower in cows fed diets that contained HMTBa, regardless of dose (57.9% vs 55.6%, P = 0.01). There were no effects of treatment on any other nutrient digestibility parameters. Overall, feeding HMTBa blended with a FA supplement had no effect on milk production, components or digestion.

## 2265T The effect of dietary fat level on mammary arteriovenous fatty acid difference and milk fat yield in Holstein cows.

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US dairy farmers invest in fatty acid (FA) supplements in attempts to increase energy intake and maximize milk fat yield, but the effect of dietary fat level on mammary FA uptake and metabolism are not well described. The objective was to characterize mammary FA arteriovenous (AV) difference and uptake efficiency (arterial concentration / AV difference) of plasma triglycerides (TG) and nonesterified FA (NEFA) at 2 levels of dietary fat. The hypothesis was that mammary AV difference of TG and NEFA would be greater in cows fed a high-fat diet compared with a low-fat diet. A secondary hypothesis was that as arterial concentration of TG and NEFA increased, efficiency of mammary extraction of FA would increase. Nine multiparous Holstein cows were arranged in a crossover design with 2, 14 d periods. Treatments were a low-fat diet (LF; ether extract formulated at 2.9% DM) or a high fat diet containing whole cottonseed and a prilled fatty acid supplement (HF; ether extract formulated at 5.4% DM). Data were analyzed in a mixed model that included the random effect of cow and period and fixed effect of treatment in JMP Pro 15. For plasma analysis, the fixed effect of time and interaction of treatment by time was added to the model. There was no effect of treatment on milk or milk protein yield. HF increased milk fat yield 88 g/d ( $P = 0.02$ ) through an increase in preformed ( $P < 0.001$ ) and mixed ( $P = 0.04$ ) source FA. HF also increased milk fat percent ( $P = 0.02$ ) and increased the percent of preformed FA ( $P < 0.001$ ) while decreasing the percent of de novo FA ( $P < 0.001$ ). There was no effect of treatment or time on mammary AV difference of TG or NEFA. There was a positive linear relationship ( $P < 0.001$ ,  $R^2 = 0.582$ ) between arterial TG concentration and mammary TG AV difference. In conclusion, treatment influenced milk fat yield, fat percent, and FA composition but did not influence mammary TG or NEFA AV differences. However, higher arterial TG concentrations resulted in a greater TG AV difference, indicating greater mammary TG uptake efficiency.

## 2127M Short-term effect of increasing dietary fatty acids on milk fat.

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Milk fat is an important component of milk pricing for US dairy farms. Fat supplements are commonly fed to increase energy balance and milk fat yield on dairy operations. However, increasing absorbed fatty acids (FA) commonly decreases de novo FA synthesis. The objective of this experiment was to determine the short-term effect of increasing dietary FA supplementation on milk fat yield and mammary de novo FA synthesis in lactating dairy cows fed a low-fat diet. The hypothesis was that FA supplementation would increase milk fat yield and decrease de novo FA synthesis in a dose-dependent manner. Twelve multiparous Holstein cows ( $2.9 \pm 0.8$  parities;  $223 \pm 29$  DIM; Mean  $\pm$  SD at start of experiment) were used in a 4x4 Latin square design with 7 d periods. Treatments were an experimental prilled free FA supplement that contained 35.6% 16:0, 54.9% 18:0, and 4.6% 18:1 (Milk Specialties Global, Eden Prairie, MN) fed at 0 (CON), 1, 2, and 3% of diet dry matter (DM). Data were analyzed in JMP Pro 15 and included the random effects of period and cow and the fixed effect of treatment. For plasma analysis, the fixed effect of time and interaction of treatment by time was added to the model. There was no effect of increasing FA supplement on milk or milk fat yield. Fatty acid supplementation quadratically increased milk fat concentration, which peaked at 2% of diet DM ( $P = 0.04$ ). Increasing dietary FA tended to linearly decrease milk de novo FA yield ( $P = 0.08$ ) and linearly decreased odd and branched-chain FA yield ( $P = 0.005$ ) while linearly increasing preformed FA yield ( $P = 0.01$ ). For milk FA profile, increasing dietary FA linearly decreased milk de novo ( $P < 0.001$ ) and odd and branched-chain ( $P < 0.001$ ) FA and linearly increased mixed ( $P = 0.002$ ) and preformed ( $P < 0.001$ ) FA percent. There was no effect of treatment, time, or their interaction on plasma nonesterified FA or plasma triglycerides. There was an effect of time on plasma glucose ( $P < 0.001$ ), but there was no effect of treatment or treatment by time interaction. In conclusion, increasing dietary FA did not change milk or milk fat yield but modified milk FA composition.

## 2376W Linking amino acids to milk fat synthesis.

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Most milk is priced based on its end use. Milk fat is one of the most important milk components considered in the establishment of the final milk price. Increased milk fat yield has been observed when dairy cows are supplemented with essential amino acids (EAA). This effect has been related to the role that EAA play in regulating mTORC1, which is associated with SREBP-1, an essential factor involved in the regulation of the de novo fatty acid synthesis. Thus, this study aims to evaluate the effects of each EAA, at physiological levels, in de novo milk fat synthesis in bovine mammary epithelial cells. Primary cells from bovine mammary gland were submitted to 12 treatments, varying EAA profile. The positive control simulate the physiological EAA concentration, in the negative control all EAA were omitted, and for the other treatments one EAA was omitted at time. For measuring the de novo milk fat synthesis, we used, as a tracer, isotopically labeled acetate. Least Squares Means (LS Means) was used for the analysis of the linear model-based data using the package 'lsmeans' version 2.30-0. Omission of L-methionine (Met), L-leucine (Leu), and L-isoleucine (Ile) decreased ( $P < 0.05$ ) the isotopic enrichment of lauric and myristic acids. Removal of these EAA were associated with reductions in de novo synthesis of both fatty acids. Synthesis of palmitic acid seemed to be more responsive with removal of Leu, L-lysine (Lys), and L-histidine (His) resulting in a significant reduction in the isotope enrichment. Omission of Met, Leu, Ile, His, and Lys influenced milk fat synthesis in the primary mammary epithelial cells subjected to physiological levels of EAA. It is important to understand the relationship between EAA and milk fat synthesis in the mammary gland to make it possible to formulate diets that can increase milk fat synthesis.



## 1088 Interaction between DGAT1 polymorphism, parity, and acetate supplementation on feeding behavior, milk synthesis, and plasma metabolites in dairy cows.

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Acetate supplementation increases milk fat, but interactions with animal-related factors are relatively unknown. The objective of this study was to characterize the interaction of acetate supplementation, genetic potential for milk fat synthesis (genomic PTA or DGAT1 SNP), and parity on milk fat synthesis. A total of 96 cows (49 multiparous and 47 primiparous) were arranged into 2 blocks of 48 in a crossover design with 14-d experimental periods and loosely housed with Calan gates. Treatments were a basal TMR as a control or sodium acetate mixed in the TMR at ~10 mol/d of acetate. Milk yield and composition and DMI were determined during the last week of each period. Blood samples were taken at 2 time points at the last day of each period. Data were analyzed with a model that included the fixed effect of treatment, DGAT1SNP, parity, block, and all their possible interaction and the random effect of period(block) and cow(block). For analysis with genomic PTA, DGAT1 was replaced with genomic PTA for milk fat percentage or yield. Variables that were measured through time were similarly analyzed with repeated measured. All models were hierarchically reduced and Studentized residuals outside of  $\pm 3$  were considered outliers and eliminated. The DGAT1 K232A SNP frequency was 45% AA and 51% KA, with 4% cows with either a KK or unimputable genotype. Acetate supplementation increased DMI by 1.7 kg/d in KA multiparous cows, but there was no difference in other types of cows (acetate  $\times$  DGAT1  $\times$  parity  $P = 0.10$ ). There was no effect of acetate on milk yield ( $P = 0.86$ ) and no interaction with DGAT1 polymorphism or parity. Acetate supplementation increased milk fat yield and concentration by 117 g/d and 0.31 percentage units, respectively ( $P < 0.001$  for both) and there was no interaction with DGAT1 polymorphism or parity. There was also no interaction of acetate and genomic PTA for milk fat yield (acetate  $P < 0.001$  both analyses). In conclusions, acetate supplementation increases milk fat synthesis regardless of parity or genetic potential for milk fat synthesis.

## 2132M Meta-analysis examining the effect of different ratios of palmitic and oleic acids in supplemental fat blends on molar changes in de novo and preformed milk fatty acids in dairy cows.

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We performed a meta-analysis to evaluate the effect of different ratios of palmitic (C16:0) and oleic (C18:1) acids in supplemental fat blends on molar yields of milk FA (mol/d) using 5 studies in early to mid-lactation cows. Treatments were control diets (CON) with no supplemental FA (n = 81) and diets supplemented at 1.5% DM with FA blends containing 80% C16:0 and 10% C18:1 (80:10, n = 56), 70% C16:0 and 20% C18:1 (70:20, n = 56), and 60% C16:0 and 30% C18:1 (60:30, n = 82). Diets (% DM) contained (mean  $\pm$  SD) 29.1  $\pm$  2.0 NDF, 28.5  $\pm$  2.8 starch, and 16.7  $\pm$  0.65 CP. Across studies, cows averaged 27.2  $\pm$  4.8 kg/d DMI, 46.9  $\pm$  2.0 kg/d milk, 1.81  $\pm$  0.18kg/d milk fat, and 1.52  $\pm$  0.07 kg/d milk protein. The statistical model included the random effect of study, cow within study, and period or day of treatment within study. Contrasts tested the overall effect of FA supplementation (FAT) versus CON, and linear and quadratic effects of increasing C18:1 in supplemental FA blends. Sources of milk FA were classified as de novo (16 carbons). Results are sequenced as: CON, 80:10, 70:20, and 60:30. Overall, FAT had no effect on de novo yield (2.39, 2.39, 2.31, and 2.30 mol/d, P = 0.46) although they increased C4:0 (P = 0.04) and decreased C10:0 and C12:0 (P < 0.01) yields. Compared with CON, FAT increased mixed (2.27, 2.65, 2.58, and 2.49 mol/d, P < 0.01) and preformed (2.09, 2.10, 2.24, and 2.27 mol/d, P = 0.04) yields including C16:0, C18:0, and C18:1 (P < 0.05). Increasing C18:1 in FA blends had no effect on de novo (P = 0.37) yield although there was a tendency to linearly increase C14:0 yield (P = 0.06). Increasing C18:1 in FA blends decreased mixed FA yield (P = 0.03) by decreasing C16:0 (P = 0.03) and increased preformed yield (P < 0.01) by linearly increasing C18:0 and C18:1 (P  $\leq$  0.06) and quadratically increasing trans C18:1 FA (P  $\leq$  0.09). Results indicate that feeding FA blends of C16:0 and C18:1 increases mixed and preformed FA yields with no effect on the overall yield of de novo FA; increasing C18:1 in FA blends linearly increases preformed FA yield.



## **Nutrient partitioning**

## 2071M The role of lactation stage on nutrient partitioning in response to acetate supply.

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Acetate is the main source of energy and carbon for milk fat synthesis in the dairy cow, and recent studies have suggested that exogenous acetate supply may stimulate energy partitioning toward milk synthesis, however, it is not known if this occurs at any time of lactation or at a specific stage. Whole-body insulin resistance is a nutrient partitioning mechanism described in lactating cows, and it is evaluated through intravenous glucose tolerance tests (IVGTT). The objective of this study was to investigate the role of lactation stage on plasma indicators of nutrient partitioning in response to acetate supply. Eight ruminally cannulated multiparous lactating Holstein cows were randomly assigned to treatments in a 2 × 2 Latin square design, repeated at 3 lactation stages (n = 8): early: 25 ± 6 DIM; peak: 60 ± 7 DIM; mid: 163 ± 19 DIM. Treatments were 5 d rumen infusion of 10 moles/d of sodium chloride (control) or 10 moles/d of sodium acetate with a 7 d washout period between treatments. On d 3 of treatments at each lactation stage, plasma samples were obtained before feeding (AM) and 6 h after feeding (PM). Glucose tolerance tests were performed on d 4 of infusion (0.25 g glucose/kg BW, IV), 60 min before feeding. Data were analyzed with the random effects of cow and period, and fixed effects of treatment, lactation stage, time and their interactions. No treatment effect or treatment interactions were observed for any response variable. A stage by time interaction was observed for plasma NEFA and BHB, with NEFA being greater in AM than PM and BHB greater in PM than in AM in early and peak, but not at mid lactation (all P < 0.05). A tendency for greater NEFA was observed in the acetate treatment as compared with control (P = 0.08). Plasma glucose was greater in mid as compared with early lactation (P < 0.05). Incremental area over the curve (AUC) at 60 and 150 min after GTT was greater in early as compared with peak and mid lactation. Overall, acetate had no major effects on plasma metabolites that represent nutrient partitioning. However, as expected for cows that are mobilizing body reserves to support lactation, greater plasma NEFA and BHB, lower glucose, and greater AUC following GTT, was observed in early lactation.



## 1303 The impact of absorbed nutrients on energy partitioning throughout lactation.

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Energy partitioning between milk and body reserves varies over a lactation. In the postpartum period, plasma insulin concentration and insulin sensitivity of tissues are low, resulting in a partitioning of absorbed nutrients and body reserves toward milk synthesis. As lactation progresses, insulin secretion and sensitivity increase, favoring deposition instead of mobilization of body reserves. Fermentation and digestion of diet components determine the temporal pattern and profile of absorbed nutrients. The pattern and profile, in turn, alter hormonal signals to affect energy partitioning differently depending on the physiological state of the cow. High starch diets increase ruminal propionate production, the flow of gluconeogenic precursors to the liver, and blood insulin concentrations. In high-producing dairy cows, the glucose produced will preferentially be used by the mammary gland for milk production. As lactation progresses and milk yield decreases, glucose will increasingly be used to replenish body reserves. Because acetate is less insulinogenic than propionate, diets with less starch and more digestible fiber that increase ruminal production of acetate relative to propionate can minimize body weight gain. High diet starch concentration and fermentability also can induce milk fat depression by increasing the production of biohydrogenation intermediates that inhibit milk fat synthesis and thus favor energy partitioning away from the mammary gland and toward body reserves. Not only carbohydrates, but also other nutrients such as supplemental fatty acids can affect energy partitioning by affecting insulin concentration and insulin sensitivity of tissues. Depending on profile, physiological state, and interactions with other nutrients, supplemental fatty acids might increase milk yield at the expense of body reserves or partition energy to body reserves at the expense of milk yield. Understanding the biology of these interactions can help nutritionists better formulate diets for cows at various stages of lactation.



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# 1377 Major changes in feed energy values and energy requirements in the 2021 NASEM Nutrient Requirements of Dairy Cattle.

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Energy supply and requirements were addressed in Chapter 3 of the 2021 NASEM Nutrient Requirements of Dairy Cattle. The net energy (NE) system was retained, but the method to estimate dietary NE content was modified. Most importantly, changes were made to feed fractions and their digestibilities and to maintenance requirements. Dietary digestible energy (DE) is calculated as the sum of digestible feed fractions, including neutral detergent fiber (NDF), starch, fatty acid, protein, and a new fraction, residual organic matter. Each fraction for each individual feed has a base digestibility. Instead of using a cow at maintenance for this base, the new system uses a cow eating a 26% starch diet at 3.5% of BW for the base. The digestibility discount with increasing intake is less than in the 2001 NRC; however, the digestibility of fiber is now decreased with increasing starch content. Thus, similar to the 2001 system, energy values can only be estimated for complete diets, not individual feeds. The conversion of DE to Metabolizable Energy (ME) depends on gas and urinary energy losses, which depend on the diet content of fat and digestible NDF and on expected protein excretion. **The conversion of ME to NE for Lactation was set at 0.66. Energy requirements for maintenance were increased by 25%,** and refinements were made to NE requirements for pregnancy, lactation, and body gain. Body gain was portioned into frame growth (true structural growth) and body condition change. Gut fill is 18% of frame gain but does not change with body condition change; thus, condition change is all body tissue, unlike in 2001. Compared with the 2001 NRC, the new system does a better job of estimating the energy supply of diets as diet composition changes and diet NE values are typically higher; in addition, NE requirements for cows are higher, and calculated feed efficiency will be lower for larger cows. Balancing diets for energy still will require more than the model. How diet composition affects intake and nutrient partitioning must be considered and monitored when feeding dairy cattle.



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