

## Ruminant Nutrition II

**256 Low and high methane emitting cows hold their ranking over different feeding strategies.** A. R. Bayat<sup>\*1</sup>, T. Luukkonen<sup>1</sup>, P. Kairenius<sup>1</sup>, H. Leskinen<sup>1</sup>, T. Hurme<sup>2</sup>, S. Ahvenjärvi<sup>1</sup>, and J. Vilkkki<sup>1</sup>, <sup>1</sup>*Green Technology, Natural Resources Institute Finland (Luke), Jokioinen, Finland*, <sup>2</sup>*Natural Resources and Bioproduction, Natural Resources Institute Finland (Luke), Jokioinen, Finland*.

To study whether the cows' ranking based on methane (CH<sub>4</sub>) emission is defined by the host animal irrespective of dietary strategy, 100 Nordic Red cows in mid-lactation were ranked according to CH<sub>4</sub>/DMI emission using respiratory chambers. Two groups of 5 low- and 5 high-emitters were selected, fitted with rumen cannulas and subjected to different diets in 3 35-d periods. High grass (HG, 70:30), low grass (LG, 30:70) and red clover (RC, 50:50) based-diets differing in forage to concentrate ratio were fed. Proc GLIMMIX of SAS was used to analyze the data with a model that included random effect of cow, fixed effects of period, diet, group, and interaction of diet and group. Intakes of DM and gross energy tended ( $P \leq 0.12$ ) to be lower for the low- compared with high-emitter cows (23.7 vs 25.5 kg/d and 421 vs 455 MJ/d). Intakes of DM and gross energy were higher ( $P < 0.01$ ) for LG compared with HG and RC (26.5 vs 23.4 and 23.7 kg/d; 476 vs 416 and 423 MJ/d). Intake of NDF was higher for HG compared with LG and RC (8.65 vs 7.95 and 7.78 kg/d;  $P < 0.01$ ). Both groups had similar milk yield. HG had lower milk yield than LG and RC (34.2 vs 39.3 and 38.4 kg/d;  $P < 0.01$ ). Digestibility of OM (71.4 vs 73.0%;  $P < 0.01$ ) and NDF (55.6 vs 59.2%;  $P < 0.05$ ) was lower for low- compared with high-emitters. Low-emitters tended ( $P = 0.08$ ) to have lower CH<sub>4</sub>/DMI than high-emitters (20.9 vs 22.5 g/kg). LG had lower CH<sub>4</sub>/DMI than HG and RC (19.7 vs 23.4 and 22.0 g/kg;  $P < 0.01$ ) and the interaction of diet and group was not significant. High-emitters consuming HG and LG had higher CH<sub>4</sub> emission (g/d) than low-emitters consuming LG (581 and 580 vs 466 g/d;  $P = 0.06$  for group  $\times$  diet interaction). Rumen DM fill, measured by rumen evacuation, did not differ between diets or groups but rumen NDF fill was lower for LG than HG (6.47 vs 7.35 kg;  $P < 0.01$ ). Rumen molar acetate to propionate ratio was lower for LG compared with HG and RC (2.76 vs 3.49 and 3.46;  $P < 0.01$ ). This ratio tended to be lower for low- than high-emitters (3.09 vs 3.39;  $P = 0.09$ ). The results suggest that CH<sub>4</sub>/DMI ranking of cows is determined by the host animal irrespective of the diet fed.

**Key Words:** methane, ranking, dairy cow

**257 Effects of feeding brown midrib dwarf pearl millet silage on lactational performance and enteric methane emission in dairy cows.** M. T. Harper<sup>\*</sup>, A. Melgar, G. Roth, and A. N. Hristov, *The Pennsylvania State University, University Park, PA*.

The objective of this experiment was to evaluate the production effects of replacing corn silage (CS; serving as the control) with brown midrib dwarf pearl millet silage (PM) in the total mixed ration of lactating dairy cows. Sixteen Holstein cows (65  $\pm$  21 DIM; BW 630  $\pm$  71 kg) were used in a replicated 2  $\times$  2 Latin square design experiment with two 28-d periods. Feeding was ad libitum for 5 to 10% refusals. The control diet consisted of (DM basis): 50% CS, 6% alfalfa haylage, 4% hay/straw mixture, and 40% concentrate feeds. For the PM diet, 20% of CS was replaced with PM (on DM basis). Control and PM diets were 16.7 and 17.2% CP, 30.3 and 32.4% NDF, and 28.0 and 24.1% starch, respectively. Metabolizable protein balance of control and PM diets was 27 and 208 g/d, respectively; NE<sub>L</sub> balance was -0.7 and -0.5 Mcal/d.

Enteric methane emission was measured using the GreenFeed system. The PM diet resulted in equal DMI as the control (29.0 vs 29.1 kg/d; SEM = 0.65,  $P = 0.78$ , respectively) but lower milk yield (49.6 vs 51.3 kg/d; SEM = 2.02,  $P < 0.001$ ) and lower feed efficiency (1.72 vs 1.77 kg/kg milk; SEM = 0.05,  $P = 0.01$ ). Energy corrected milk (ECM) yield (46.7 kg/d; SEM = 1.92,  $P = 0.86$ ), and ECM feed efficiency were not different between diets. The PM diet tended to increase milk fat content compared with the control diet (3.71 vs 3.47%; SEM = 0.118,  $P = 0.06$ , respectively) but true protein and lactose content were not affected. Yields of the individual milk components were not affected ( $P \geq 0.23$ ) by diet and averaged 1.81 kg/d fat, 1.45 kg/d true protein and 2.51 kg/d lactose. Enteric methane emission was increased by the PM diet over the control (454 vs 396 g/d; SEM = 18.4,  $P < 0.001$ ) as was methane yield (15.7 vs 13.8 g/DMI; SEM = 0.54,  $P = 0.001$ ) and methane intensity (9.6 vs 8.3 g/kg ECM; SEM = 0.39,  $P = 0.001$ ). Brown midrib dwarf pearl millet silage has potential to partially replace CS in the diet of dairy cattle without affecting ECM yield and milk components. This replacement, however, is likely to increase enteric methane emission.

**Key Words:** pearl millet, methane, dairy cow

**258 Assessing the potential of 3-nitrooxypropanol and canola oil alone and in combination to lower methane emissions from cattle and reduce their contribution to climate change.** M. L. Smith<sup>\*1</sup>, S. M. Duval<sup>2</sup>, M. Kindermann<sup>3</sup>, K. A. Beauchemin<sup>4</sup>, and L. Kung Jr.<sup>1</sup>, <sup>1</sup>*University of Delaware, Newark, DE*, <sup>2</sup>*DSM Nutritional Products France, Saint Louis Cedex, France*, <sup>3</sup>*DSM Nutritional Products, Basel, Switzerland*, <sup>4</sup>*Agriculture and Agri-Food Canada, Lethbridge, AB, Canada*.

The objective of this study was to assess the potential of 3-nitrooxypropanol, a novel methane inhibitor; and canola oil, a known methane mitigant; alone and in combination on methane emissions, rumen fermentation, and diet digestibility. Eight ruminally cannulated beef heifers (Angus cross, 732  $\pm$  43 kg) were used in a double 4  $\times$  4 Latin square design with 4 28-d periods and assigned to one of 4 dietary treatments. The dietary treatments were: 1) control (CON) (no supplementation of 3-nitrooxypropanol or canola oil), 2) canola oil alone (OIL) (5% of diet DM), 3) 3-nitrooxypropanol alone (NOP; 200 mg/kg of diet DM; DSM Nutritional Products Ltd., Kaiseraugst, Switzerland), and 4) 3-nitrooxypropanol and canola oil combined (NOP+OIL). After a 14-d diet adaption, dry matter intake (DMI) was recorded daily. Rumen contents were collected on d 14 and 17 for volatile fatty acid (VFA) analysis and protozoal populations. Enteric methane emissions were measured on d 18 to 21 using open circuit chambers. Diet digestibility was measured on d 24 to 27. Methane production was reduced from 26.2 (CON) to 19.6, 17.9, and 12.7 g/kg of DMI, for OIL, NOP, and NOP+OIL, respectively ( $P < 0.01$ ). Total VFA concentrations (mM) were greatest for CON (101.3), similar between OIL (94.8) and NOP (94.8), and lowest for NOP+OIL (88.3) ( $P < 0.01$ ). A decrease in acetate (A) and increase in propionate (P) proportions, and therefore a decrease in the A:P ratio was also observed with the OIL, NOP, and NOP+OIL treatments compared with CON ( $P < 0.01$ ). The OIL and NOP+OIL treatments had a reduction in protozoa counts and a reduction in DM, OM, NDF, and ADF digestibilities when compared with CON and NOP (4.43  $\times$  10<sup>4</sup> vs. 4.24  $\times$  10<sup>5</sup>/ mL rumen fluid; 60.7 vs. 66.8%; 62.0 vs. 68.7%; 47.6 vs. 61.0%; and 46.5 vs. 60.0%, respectively) ( $P < 0.01$ ). The data demonstrated that the addition of OIL and NOP are effective

means of decreasing methane production, and the combination of both caused the greatest reduction of methane emissions in cattle.

**Key Words:** 3-nitrooxypropanol, canola oil, methane

**259 Effect of pH and 22:6n-3 on in vitro biohydrogenation of 18:2n-6 by different ratios of *Butyrivibrio fibrisolvens* to *Propionibacterium acnes*.** L. Dewanckele\*, B. Vlaeminck, J. Jeyanathan, and V. Fievez, *Laboratory for Animal Nutrition and Animal Product Quality, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium.*

The objective of this in vitro study was to examine the *trans*-10 (*t*<sub>10</sub>) shift in relation to the ratio between hydrogenating bacteria capable of producing either *cis*-9,*trans*-11 conjugated linoleic acid (*c9t11* CLA) or *t*<sub>10</sub>,*cis*-12 CLA (*t10c12* CLA). The influence of the in vitro condition on this shift was also investigated. *Butyrivibrio fibrisolvens* D1 (BF) and *Propionibacterium acnes* DSM 1897 (PA) were chosen as model organisms for the production of *c9t11* CLA and *t10c12* CLA, respectively. Different ratios of these bacteria (100/0, 50/50, 10/90, 2/98, 0.4/99.6, 0/100) were incubated in different growth media containing 40 µg/mL 18:2n-6 (LA): (1) control, (2) low pH, (3) 22:6n-3 (DHA) enriched media. The low pH medium was prepared by adding 2 M HCl to the control to reduce the pH from 6.5 to 5.5. The DHA enriched medium was the control supplemented with 40 µg/mL DHA. Under control conditions, the residual amount of LA after 24 h of incubation increased with increasing amounts of PA at inoculation ( $P = 0.013$ ), which implies a lower rate of LA metabolism by the latter as compared with BF. Increasing amounts of PA also increased *t10c12* CLA accumulation ( $P = 0.002$ ) at the expense of *c9t11* CLA ( $P = 0.006$ ), with a *t*<sub>10</sub> shift, defined as  $t_{10}/t_{11} \geq 1$ , occurring when PA represented between 90% and 98% of the inoculum. The required relative amount of PA at inoculation to induce a *t*<sub>10</sub> shift decreased to 50% and 90% in the low pH or DHA enriched medium, respectively. Low pH or DHA addition did not stimulate *t10c12* CLA formation, but inhibited CLA formation by both bacteria whereby PA seemed to be more tolerant. The current results suggest that besides a specific balance between BF and PA, specific external factors might influence the *t*<sub>11</sub> to *t*<sub>10</sub> shift. A low pH or, to a lesser extent, addition of DHA gives some advantage to PA compared with BF. Nevertheless, required proportions of PA remained high under all conditions. Hence, it is unlikely that PA is the only or predominant species involved in the *t*<sub>11</sub> to *t*<sub>10</sub> shift under in vivo circumstances.

**Key Words:** biohydrogenation, linoleic acid, *trans*-11 to *trans*-10 shift

**260 Are EPA, DPA, and DHA equally effective to modulate ruminal biohydrogenation in cows? A comparative in vitro study.** P. G. Toral\*<sup>1</sup>, G. Hervás<sup>1</sup>, D. Carreño<sup>1</sup>, H. Leskinen<sup>2</sup>, A. Belenguer<sup>1</sup>, K. J. Shingfield<sup>3</sup>, and P. Frutos<sup>1</sup>, <sup>1</sup>*Instituto de Ganadería de Montaña (CSIC-Universidad de León), Grulleros, León, Spain,* <sup>2</sup>*Natural Resources Institute Finland (LUKE), Green Technology, Nutritional Physiology, Jokioinen, Finland,* <sup>3</sup>*Institute of Biological, Environmental and Rural Sciences, Animal and Microbial Sciences, Aberystwyth University, Aberystwyth, United Kingdom.*

Marine lipid supplements are rich in very long chain n-3 polyunsaturated fatty acids (PUFA) that inhibit the ruminal saturation of *trans*-11 18:1 and, consequently, may enhance the concentration of *cis*-9,*trans*-11 conjugated linoleic acid (CLA) in milk and meat. In this regard, docosahexaenoic acid (DHA, 22:6n-3) has been suggested to increase total *trans*-18:1 accumulation in the rumen to a greater extent than

eicosapentaenoic acid (EPA, 20:5n-3), but information about changes in individual 18:1 isomers is very limited. Furthermore, although EPA and DHA are accepted to be the main responsible for this modulatory effect on ruminal biohydrogenation (BH), the contribution of docosapentaenoic acid (DPA, 22:5n-3), the third most abundant n-3 PUFA in marine lipids, remains unknown. The aim of this study was to compare the impact of EPA, DPA and DHA on the BH of dietary C18 fatty acids, using batch cultures of rumen microorganisms and cannulated cows as inocula donors. The 3 PUFA were added at a dose of 2% of incubated substrate (the TMR fed to the animals; 50:50 forage concentrate ratio) and effects were examined after 24 h of incubation. Data were subjected to ANOVA using the MIXED procedure of SAS 9.4. Overall, EPA and DHA were equally effective to increase the concentration of *trans*-11 18:1 (on average, +79% compared with the control;  $P < 0.001$ ), suggesting that supplements containing differing EPA/DHA ratios (e.g., fish oils or marine algae) would have comparable effects at the same PUFA level. However, DHA further promoted alternative BH pathways that lead to *trans*-10 18:1 accumulation (+205% relative to the control;  $P < 0.01$ ). The saturation of *cis*-18:1 and non-conjugated 18:2 isomers was also constrained, particularly by DHA in the former case and by EPA in the latter. Increases in *trans*-11 *cis*-15 + *trans*-10,*cis*-15 18:2 and in *trans*-9,*trans*-14 18:2 ( $P < 0.001$ ) may indicate that EPA had specific effects on 18:3n-3 metabolism. Only minor variations in ruminal BH intermediates were observed in response to DPA (e.g., increments in *trans*-10,*trans*-13 and *cis*-15 18:1;  $P < 0.05$ ), which suggests a low contribution of this PUFA to the action of marine lipids.

**Key Words:** rumen, lipid metabolism, *trans* fatty acid

**261 Altering the ratio of dietary C16:0 and *cis*-9 C18:1 interacts with production level in dairy cows: Effects on production responses and energy partitioning.** J. de Souza\* and A. L. Lock, *Michigan State University, East Lansing, MI.*

We evaluated the effects of altering the dietary ratio of C16:0 and *cis*-9 C18:1 on production responses and energy partitioning of lactating dairy cows. Cows were blocked by milk yield and assigned to 3 groups (12 cows per group) in a main plot. Production groups were: a) low (45.2 ± 1.7 kg/d); b) medium (53.0 ± 1.6 kg/d); and c) high (60.0 ± 1.9 kg/d). Within each production group, a truncated Latin square arrangement of FA treatments was used in 2 consecutive 35 d periods. The FA treatments supplemented at 1.5% diet DM were: 1) 80:10 (80% C16:0 + 10% *cis*-9 C18:1); 2) 73:17 (73% C16:0 + 17% *cis*-9 C18:1); 3) 66:24 (66% C16:0 + 24% *cis*-9 C18:1); and 4) 60:30 (60% C16:0 + 30% *cis*-9 C18:1). The statistical model included the random effect of cow within production group, and the fixed effect of treatment, production group, period, and their interactions. Treatment by production group interactions were observed for milk yield ( $P = 0.09$ ), FCM ( $P = 0.05$ ), ECM ( $P = 0.05$ ), milk fat yield ( $P = 0.02$ ), milk protein yield ( $P = 0.06$ ), milk lactose yield ( $P = 0.08$ ), and energy partitioned to milk ( $P = 0.02$ ). Increasing *cis*-9 C18:1 in FA treatments reduced FCM, ECM, and milk energy output in low producing cows (linear,  $P < 0.05$ ), but increased these in high producing cows (linear,  $P < 0.01$ ). Increasing *cis*-9 C18:1 in FA treatments tended to reduce milk fat yield in low producing cows (linear,  $P < 0.10$ ), but increased it in high producing cows (linear,  $P < 0.01$ ). Increasing *cis*-9 C18:1 in FA treatments did not impact milk yield, milk protein yield, and milk lactose yield in low and medium producing cows, but increased these in high producing cows (linear,  $P < 0.01$ ). Regardless of production level, increasing *cis*-9 C18:1 in FA treatments increased BW change (quadratic,  $P = 0.02$ ) and BCS change (linear,  $P < 0.01$ ); however, there was no effect of treatments on DMI ( $P = 0.98$ ). Our results demonstrate that high producing dairy cows respond better

to fat supplements containing more *cis*-9 C18:1, while lower producing cows respond better to supplements containing more C16:0.

**Key Words:** energy partitioning, oleic acid, palmitic acid

**262 Effects of timing of C16:0 supplementation on production and metabolic responses of early lactation dairy cows.** J. de Souza\* and A. L. Lock, *Michigan State University, East Lansing, MI.*

Fifty-two multiparous cows were used in a randomized complete block design experiment and assigned to either a control diet containing no supplemental fat (CON) or a C16:0 supplemented diet (PA) that was fed either from calving to 24 DIM (fresh period) or from 25 to 67 DIM (peak period). Fresh diets were formulated to contain (% DM) 17% CP, 30% NDF, 24% forage NDF, and 24% starch. Peak diets were formulated to contain (% DM) 17% CP, 29% NDF, 19% forage NDF, and 26% starch. The C16:0 supplement (85% C16:0), added at 1.5% of diet DM, replaced soyhulls in the CON diets. During the fresh period, PA did not affect DMI (21.5 vs. 21.6 kg/d,  $P = 0.92$ ) or milk yield (47.7 vs. 46.3 kg/d,  $P = 0.38$ ) compared with CON. In contrast, compared with CON, PA increased milk fat content (4.89 vs. 4.48%,  $P = 0.01$ ) and yield (2.29 vs. 2.01 kg/d,  $P < 0.01$ ), milk protein yield (1.60 vs. 1.53 kg/d,  $P = 0.03$ ), and ECM (56.6 vs. 51.9 kg/d,  $P = 0.02$ ). PA decreased BW (668 vs. 709 kg,  $P = 0.05$ ), BCS (3.25 vs. 3.34,  $P = 0.04$ ), plasma insulin (0.21 vs. 0.24 ug/L,  $P = 0.05$ ), and increased plasma NEFA (0.65 vs. 0.59 mEq/L,  $P = 0.03$ ) compared with CON. A treatment by day interaction was detected for BW ( $P = 0.05$ ) and BCS ( $P = 0.07$ ) due to PA only decreasing these variables after 10 DIM compared with CON. During the peak period, compared with CON, PA did not affect DMI (29.9 vs. 30.2 kg/d,  $P = 0.68$ ), but increased milk yield (58.0 vs. 54.6 kg/d,  $P = 0.01$ ), milk fat content (3.88 vs. 3.67%,  $P < 0.01$ ) and yield (2.27 vs. 2.06 kg/d,  $P < 0.01$ ), milk protein yield (1.80 vs. 1.66 kg/d,  $P = 0.04$ ), and ECM (62.3 vs. 57.8 kg/d,  $P < 0.01$ ). Compared with CON, PA reduced plasma insulin concentration (0.25 vs. 0.32 ug/L,  $P = 0.05$ ), but did not affect plasma NEFA concentration (0.35 vs. 0.32 mEq/L,  $P = 0.41$ ) or BW (673 vs. 684 kg,  $P = 0.93$ ). There were no interactions between feeding PA and the time that supplementation started for production variables. Our results demonstrate that supplementing C16:0 during early lactation increases ECM without changes in DMI. Feeding PA may increase BW loss during the fresh period, but not during the peak period.

**Key Words:** body condition, palmitic acid, postpartum

**263 Abomasal infusion with an exogenous emulsifier improves fatty acid digestibility and milk fat yield of lactating dairy cows.** J. de Souza\*, M. M. Western, and A. L. Lock, *Michigan State University, East Lansing, MI.*

We evaluated the effects of abomasal infusion of an emulsifier (Polysorbate 80, Tween 80) on fatty acid (FA) digestibility and production responses of lactating dairy cows. Eight rumen-cannulated cows (109 ± 18 DIM) were randomly assigned to a treatment sequence in replicated 4 × 4 Latin squares with 18-d periods including 7 d of washout, and 11 d of infusion with sampling on the last 4 d. Treatments were abomasal infusions of water carrier only (CON) and 3 levels of increasing doses of Tween 80 delivering 15 (D-15), 30 (D-30), and 45 (D-45) g/d. The Tween 80 was dissolved in water before infusions, which were delivered at 6-h intervals. Cows were fed the same diet which contained (% DM) 31% NDF, 17% CP, 25% starch and 4% FA (2% DM from a saturated FA supplement containing 33% C16:0 and 51% C18:0). The statistical

model included the random effect of cow within square, and the fixed effect of treatment, period, square, and their interactions. Results in the text are presented in the following sequence: CON, D-15, D-30 and D-45. Increasing Tween 80 infusion quadratically increased apparent total-tract digestibility of total FA (60.7, 65.3, 70.9, and 66.8%,  $P = 0.05$ ), 16-carbon FA (61.7, 63.9, 70.4, and 66.7%,  $P = 0.04$ ), and 18-carbon FA (59.8, 65.6, 71.1, and 66.6%,  $P = 0.04$ ). Increasing Tween 80 quadratically increased absorbed total FA (625, 670, 744, 658 g/d,  $P = 0.01$ ), 16-carbon FA (151, 157, 197, and 157 g/d, quadratic,  $P = 0.04$ ), and 18-carbon FA (420, 460, 500, 444 g/d,  $P = 0.01$ ). The D-45 treatment tended to reduce DMI compared with the other treatments (29.0, 28.8, 29.6, and 27.6 kg/d, quadratic,  $P = 0.09$ ). Increasing Tween 80 infusion quadratically increased milk fat content (3.23, 3.35, 3.45, and 3.35%,  $P = 0.03$ ), milk fat yield (1.54, 1.61, 1.65, and 1.55 kg/d,  $P = 0.02$ ), ECM (45.7, 46.9, 47.5, and 45.3 kg/d,  $P = 0.03$ ), and plasma NEFA concentration (95.6, 98.4, 101.2, and 98.6 μEq/L,  $P = 0.05$ ). There was no effect of treatments on milk yield (47.9, 48.3, 48.0, and 46.6 kg/d,  $P = 0.12$ ). In conclusion, infusing an exogenous emulsifier improved FA digestibility and milk fat yield responses when cows were fed a diet containing a saturated FA supplement.

**Key Words:** fatty acid digestibility, emulsifier, milk fat

**264 Milk fat depression in dairy ewes fed marine lipids: What are the reasons behind individual variation?** P. G. Toral\*<sup>1</sup>, L. Rodríguez-López<sup>1</sup>, G. Hervás<sup>1</sup>, A. K. K. Salama<sup>2</sup>, G. Caja<sup>2</sup>, and P. Frutos<sup>1</sup>, <sup>1</sup>*Instituto de Ganadería de Montaña (CSIC-Universidad de León), Finca Marzanas s/n, Grulleros, León, Spain*, <sup>2</sup>*Grup de Recerca en Remugants (G<sup>2</sup>R), Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.*

Dairy ewes are less prone than cows to milk fat depression (MFD) but suffer this syndrome when marine lipids are added to their diet to improve milk fatty acid (FA) composition. This is very detrimental as most ovine milk is used for cheese manufacture. However, there are large individual differences in MFD severity; the reasons behind this variability being uncertain. This study was conducted in lactating sheep to test the hypothesis that differences in milk concentration of antilipogenic FA or in the transcriptional regulation of mammary lipogenesis may account for that individual variation. We used 15 ewes receiving, for 35 d, a total mixed ration supplemented with 0 (control; n = 5) or 20 g of fish-oil/kg DM [10 animals were selected out of 22 and divided in those showing a strong (RESPON+, n = 5) or slight (RESPON-, n = 5) MFD]. Milk production and composition, and milk FA profile were recorded for 3 consecutive days before and after treatments. Candidate gene expression was analyzed by quantitative reverse transcription-PCR on mRNA isolated from milk somatic cells collected before (d -2 or -1) and after (d 34 or 35) the dietary treatments. Data were analyzed with the MIXED procedure of SAS 9.4 using orthogonal contrasts. Milk production was not affected by the diets ( $P > 0.10$ ) but milk fat concentration decreased by 25.4% in RESPON+ and 7.6% in RESPON- ( $P < 0.001$ ). Supplementation with fish oil enhanced ( $P < 0.01$ ) the milk content of both potentially healthy FA (e.g., *cis*-9,*trans*-11 CLA, *trans*-11 18:1, or very long chain n-3 FA) and antilipogenic FA (e.g., *cis*-9 16:1, *trans*-10 and *cis*-11 18:1, *trans*-10,*cis*-15 18:2, *trans*-9,*cis*-11 and *trans*-10,*cis*-12 CLA, and 10-oxo-18:0). Nevertheless, differences between RESPON- and RESPON+ were hardly detected. Consumption of the MFD-inducing diet was accompanied by reductions in the mRNA abundance of *ACSS2*, *FASN*, *LPIN1* and *INSIG1* ( $P < 0.10$ ), but only *SCD* and *GPAT4* tended to differ between RESPON+ and RESPON- ( $P < 0.10$ ). These results oblige to reject the hypothesis and conduct a

thorough evaluation of individual responses at systemic, ruminal, and mammary levels to explain the individual variation in MFD severity.

**Key Words:** antilipogenic fatty acid, gene expression, nutrigenomics

**265 Effects of supplementation of oleic acid and stearic acid in low fat and high fat diets on milk performance of early lactation cows.** Y. T. Chen<sup>\*1</sup>, G. L. MA<sup>1</sup>, J. H. Harrison<sup>2</sup>, and E. Block<sup>3</sup>, <sup>1</sup>Washington State University, Pullman, WA, <sup>2</sup>Washington State University, Puyallup, WA, <sup>3</sup>Church and Dwight Animal Nutrition, Princeton, NJ.

Two experiments were conducted to study the effects of supplementation of rumen inert C18:1 (Megalac) and C18:0 (Energy Booster) in diets containing low fat (3.3% DM, experiment 1) and high fat (6.6% DM, experiment 2) on milk performance in early lactation cows. The high fat diets were formulated by replacement of 2% barely grain in low fat diet with soybean oil, and the contents of other ingredients were similar. Each experiment utilized 30 cows blocked by parity and predicted transmitting ability, and randomly fed diets either supplemented with rumen inert C18:1 or C18:0 from 3 to 14 wk after calving. Milk yield and DMI were recorded daily, and milk samples were collected weekly. Data were analyzed using a randomly blocked design with repeated measurements. When cows were fed diets containing 3.3% fat, the cows fed C18:0 supplement had more ( $P < 0.05$ ) DMI by 2.4 kg/d, milk fat percentage by 0.3% unit, milk fat yield by 0.2 kg/d, milk protein yield by 0.07% unit, milk protein percentage by 0.1% unit, milk lactose percentage by 0.1%. The milk production efficiency (milk yield/DMI) of cows fed C18:1 supplement was greater ( $P < 0.05$ ) than cows fed C18:0 supplement (1.9 vs 1.8). The milk yield, other milk components percentages and yields, net energy balance and BCS were similar. In milk, cows fed C18:1 supplement had greater ( $P < 0.05$ ) concentrations of C18:1 cis-9, and cis-6, and cows fed C18:0 supplement had greater concentration of C18:0. In blood, the concentration of  $\beta$ -hydroxybutyrate was greater ( $P < 0.05$ ) in cows fed C18:0 supplement. In experiment 2, the milk protein percentage of cows fed C18:0 supplement was greater ( $P < 0.05$ ) than cows fed C18:1 supplement, while the DMI, milk yield, percentage and yield of other milk components were not affected. The milk production efficiency (milk yield/DMI) of cows fed C18:1 supplement was greater ( $P < 0.05$ ) than cows fed C18:0 supplement (2.0 vs 1.9). The results of these studies suggest that the milk performance of early lactation cows supplemented with rumen inert FA varies due to source of inert fat and amount of basal fat in the diet.

**Key Words:** oleic acid, linoleic acid, milk synthesis

**266 Body temperature of corn- and wheat-fed dairy cows.** J. B. Garner, S. R. O. Williams, P. J. Moate, J. L. Jacobs, M. J. Auld<sup>dist\*</sup>, and W. J. Wales, Dairy Production Sciences, Agriculture Research Division, Department of Economic Development Jobs Transport and Resources, Ellinbank, Victoria, Australia.

The effects of nutritional strategies on body temperature of dairy cows are not well understood but could play an important role in adapting cows to changing climates. Cereal grains including wheat grain are rapidly degraded in the rumen while corn has a slower rate of fermentation. There is preliminary data that indicates there may be differences in body temperature between cows fed wheat or corn-grain. Twenty-four dairy cows were fed a diet that included either wheat grain (12 cows) or corn grain (12 cows) at 430 g/kg DM offered. Half the daily ration was offered at each of 06:00 and 15:00. At wk 4, 10 and 16 cows entered individual controlled-climate chambers at thermoneutral conditions

for 48 h. Intravaginal temperature was recorded every 15 min using indwelling loggers. At wk 4, duration between afternoon feeding and maximum temperature tended ( $P < 0.10$ ) to be longer for cows fed wheat (245 min) than cows fed corn (128 min). At wk 10, the maximum temperature after the morning feeding was lower ( $P < 0.05$ ) in cows fed wheat (38.7°C) than cows fed corn (38.9°C). Cows fed wheat also had a lower minimum temperature than cows fed corn. After combining data across the entire experiment, the duration between feeding and maximum temperature was greater ( $P < 0.05$ ) for cows fed wheat (230 min) than cows fed corn (155 min). Maximum temperature after the morning feeding was lower ( $P < 0.05$ ) in cows fed wheat (38.6°C) than cows fed corn (38.8°C). Daily minimum temperature was lower ( $P < 0.05$ ) in cows fed wheat (38.0°C) than those fed corn (38.1°C). Reasons for these differences, which are in contrast to the ruminal degradation rates of wheat and corn, are unclear. In our previous research, acetate concentrations in the ruminal fluid of corn-fed cows have been greater than in wheat-fed cows. Diets that induce acetate fermentation in the rumen have previously been reported to increase body temperatures in cattle, and we speculate that this may have been the cause for the higher body temperatures of the corn-fed cows in this experiment. Irrespective of the mechanisms for the differences in body temperature, the feeding of different types of grains constitutes a potential nutritional strategy for ameliorating heat stress in dairy cattle.

**Key Words:** diet-induced thermogenesis, thermoregulation, rumen fermentation

**267 Heat stress decreases transcription of protein metabolism-related genes in mammary tissue of middle lactating cows.** D. P. Bu<sup>\*1,5</sup>, L. Ma<sup>1,4</sup>, S. T. Gao<sup>1</sup>, L. H. Baumgard<sup>2</sup>, and M. Bionaz<sup>3</sup>, <sup>1</sup>State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, <sup>2</sup>Department of Animal Science, Iowa State University, Ames, IA, <sup>3</sup>Animal and Rangeland Sciences, Oregon State University, Corvallis, OR, <sup>4</sup>CAAS-ICRAF Joint Lab on Agroforestry and Sustainable Animal Husbandry, World Agroforestry Centre, East and Central Asia, Beijing, China, <sup>5</sup>Hunan Co-Innovation, Changsha, Hunan, China.

Heat stress decreases milk production, compromises animal health, and increases mortality. Study objectives were to understand the biological adaptation of mammary tissue to heat stress via analysis of the transcriptome using RNA-seq. Four multiparous Holstein dairy cows (101  $\pm$  10 DIM; 574  $\pm$  36 kg BW, 38  $\pm$  2 kg milk/d) were randomly assigned to 1 of 4 environment chambers with a crossover design. Following a 9d adaptation period, cows were either subjected to heat stress [HS: 36°C during the day and 32°C during the night; THI = 87.2 and 81.8] for 9d or kept in thermal neutral conditions [20°C; THI = 65.5] for 9d, but pair-fed (PF) with heat-stressed cows. There was a 30d washout period between periods. Mammary biopsies were obtained at the end of each period. HS decreased milk yield (17%) and protein content (4.1%). HiSeq2000 platform was used to measure the mRNA profile. Data were normalized by Lowess prior ANOVA analysis using JMP Genomic (SAS systems) with treatment as the main effect and cow as the random effect. HS had a minor effect on the transcriptome with only 198 differentially expressed genes (DEG; FDR < 0.05; 1.4% of annotated genes measured): 53 upregulated and 145 downregulated genes in HS vs. PF. No gene known to be related to milk or protein synthesis was affected by treatment. Functional analysis was performed using the Dynamic Impact Approach and Database for Annotation, Visualization and Integrated Discovery revealed general inhibition of pathways in HS vs. PF, in particular, pathways affected were protein export, proteasome,

basal transcription factors, and steroid biosynthesis. The inhibition of basal transcription factors and protein export may partly explain the observed decrease in milk and protein synthesis. The biological significance of inhibited mammary steroid biosynthesis remains unclear; however, decreased steroidogenesis has been previously reported in

heat-stressed bovine granulosa cells. In summary, transcriptome analysis revealed that HS inhibited metabolic activity by decreasing transcripts associated with protein export that might affect lactation performance.

**Key Words:** transcriptomics, mammary tissue, heat stress