

## RUMINANT NUTRITION VII

### 0667 Effect of reduced energy density of close-up diet on ruminal fermentation parameters in multiparous Holstein cows.

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The objective of this study was to determine the effect of reduced energy density of close-up diet on ruminal fermentation parameters in multiparous Holstein cows. Fourteen dry cows were utilized in a randomized block design. Cows were blocked by their milk production in the first 3 mo of the previous parity, BW, and expected calving date and assigned randomly into one of three energy levels (6.8, 6.2, 5.4 MJ of NE<sub>L</sub>/kg; 14.0% CP) diets to meet 100% (100NRC; *n* = 4), 90% (90NRC; *n* = 5), 80% (80NRC; *n* = 5) of the NRC (2001) dietary NE<sub>L</sub> recommendation, respectively, from 21 d before expected day of calving. After parturition, all cows were fed the same lactation diet to 28 d in milk (DIM). Intake of DM (DMI) of individual cows was determined every day. Ruminal fluid was obtained at 1030 h on -21, -14, -7, 1, 3, 5, 7, 14, 21, and 28 d relative to parturition, and at 0830, 1030, 1230, 1430, 1630, and 1830 h on 7 DIM, at 0930, 1130, 1330, 1530, 1730, and 1930 h on 8 DIM. Data were analyzed by SPSS with repeated measures procedure. The reduced energy density diet decreased the average DMI prepartum (*P* < 0.05) and tended to increase the DMI postpartum (*P* = 0.08). The average ruminal pH of the 80NRC group was higher prepartum (6.88, 6.35, 6.44; *P* < 0.05) and lower (6.05, 6.21, 6.30; *P* < 0.05) during the first 4 wk of lactation compared with 90NRC and 100NRC groups. The 80NRC group had three sampling points of pH below 5.6 on 7 and 8 DIM, none for 90NRC and 100NRC groups. The reduced energy density diet depressed the average ruminal concentration of propionate (16.7, 17.9, 19.6 mmol/L; *P* = 0.04), tended to decrease butyrate (7.4, 9.4,

10.4 mmol/L; *P* = 0.06) prepartum, and increased the average concentration of total VFA (112.6, 95.8, 92.0 mmol/L; *P* < 0.001) and decreased the ratio of acetate to propionate (2.4, 2.3, 2.7; *P* = 0.03) during the first 4 wk of lactation. In conclusion, the cows fed reduced energy density diet prepartum had higher VFA concentration for energy metabolism but were more susceptible to subacute ruminal acidosis postpartum.

**Key Words:** Holstein cow, dietary energy density, ruminal parameters

### 0668 Prepartum dietary energy strategies for Holstein dairy cows: Effects on markers of negative energy balance and performance.

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Study objectives were to compare different dry cow nutritional strategies and their influence on periparturient concentrations of β-hydroxybutyrate (BHBA) and nonesterified fatty acids (NEFA) as analytes related to negative energy balance and periparturient dry matter intake (DMI) and milk yield. Cows (*n* = 84) were dried off 57 d before expected calving and assigned to one of three diets: a controlled-energy diet (L) that was formulated to meet, but not greatly exceed, energy requirements for the entire dry period; a step-up approach (I) in which animals received diet L for the first 4 wk of the dry period and after that a diet supplying 125% of energy requirements until calving; and a higher energy diet (H) formulated to contain 150% of requirements for the entire dry period. Data measured over time were subjected to repeated measures ANOVA using PROC MIXED with treatment, time and parity as fixed effects (Table 0668). Energy content was 1.28, 1.35, and 1.42 Mcal NE<sub>L</sub>/kg DM for the L, I, and H diets, respectively. Animals fed L had lower concentrations of BHBA during both the prepartum and postpartum periods than cows fed H. Concentration of NEFA in cows fed L was higher prepartum compared with the

**Table 0668.** Least squares means for energy metabolites, DMI, and milk yield

Parameter		Treatment			Fixed effects	
		L	I	H	Treatment	Treatment x Time
		LS means ± SE			<i>P</i>	
BHBA, mmol/dL	prepartum	0.29 ± 0.01 <sup>a</sup>	0.30 ± 0.01 <sup>ab</sup>	0.34 ± 0.01 <sup>b</sup>	0.04	0.03
	postpartum	0.63 ± 0.06 <sup>a</sup>	0.77 ± 0.06 <sup>ab</sup>	0.85 ± 0.06 <sup>b</sup>	0.05	0.19
NEFA, uEq/L	prepartum	237.1 ± 12.4 <sup>a</sup>	179.5 ± 12.7 <sup>b</sup>	175.4 ± 12.5 <sup>b</sup>	0.001	0.03
	postpartum	659.1 ± 36.4 <sup>a</sup>	664.6 ± 36.7 <sup>a</sup>	795.7 ± 39.5 <sup>b</sup>	0.02	0.37
DMI, kg/d	prepartum	14.2 ± 0.3 <sup>a</sup>	15.3 ± 0.3 <sup>b</sup>	16.4 ± 0.3 <sup>c</sup>	<0.0001	0.03
	postpartum	22.3 ± 0.6	22.4 ± 0.6	22.4 ± 0.6	0.99	0.75
Milk yield, kg/d		43.8 ± 1.2	43.6 ± 1.2	43.9 ± 1.2	0.98	0.31
ECM yield, kg/d		46.1 ± 1.2	47.0 ± 1.2	48.3 ± 1.3	0.48	0.94

<sup>abc</sup>Row means with different superscripts differ.

other two treatments, while being lower in cows fed L or I compared with H postpartum. Yields of milk and energy-corrected milk (ECM) were not different among treatments. Although sample size was not sufficient to study health outcomes, results suggest positive effects on metabolic health by feeding a controlled energy diet during the dry period.

**Key Words:** energy, peripartal, controlled

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**0669 Hepatic acetyl CoA concentration decreases following feeding in early-postpartum but not in late-lactation dairy cows.** P. Piantoni\*, C. M. Ylloja, and M. S. Allen, *Michigan State University, East Lansing.*

The relationship between hepatic acetyl CoA concentration (Ac-CoA) and dry matter intake (DMI) was determined using 28 multiparous Holstein cows. Fourteen were early-postpartum (PP;  $12.6 \pm 3.8$  DIM), and 14 were late-lactation cows (LL;  $271 \pm 29.6$  DIM). Cows were fed once daily, and DMI was determined for the first 4 h after feeding. Liver and blood samples were collected before feeding and 4 h after feeding. Feed intake over the 4-h period ranged from 3.2 to 9.6 kg DM and did not differ by stage of lactation. Before feeding, Ac-CoA was greater ( $P < 0.0001$ ) for PP (mean: 36.0; range: 12.7 to 56.1 nmol/g) compared with LL (mean: 14.1; range: 7.7 to 19.4 nmol/g), and tended to decrease over the 4 h after feeding for PP only ( $P < 0.10$ ). The range of change in Ac-CoA over the 4-h period was wide for both PP (-24.3 to 10.4 nmol/g) and LL (-5.7 to 16.1 nmol/g), and was negatively related to DMI at 4 h for PP ( $P < 0.01$ ), but not for LL. The reduction in plasma NEFA concentration over the 4-h period was greater for PP than LL (-684 vs. -52  $\mu\text{Eq/L}$ ;  $P < 0.0001$ ), and was greater for PP ( $P < 0.05$ ) and tended to be greater for LL ( $P < 0.10$ ) as DMI at 4 h increased. Greater reductions in Ac-CoA were related to higher DMI during the first 4 h after feeding in PP ( $P < 0.01$ ), which is contrary to our expectation if oxidation of Ac-CoA increased hepatic energy charge. However, hepatic energy charge is dependent on the relative rates of production and utilization of high-energy phosphate bonds and the rate of utilization might have been greater for cows with higher DMI. Alternatively, increased DMI over the first 4 h after feeding might have been from decreased oxidation of Ac-CoA during meals if the greater reduction in plasma NEFA concentration reduced Ac-CoA production by  $\beta$ -oxidation. Consistent with this is that the change in Ac-CoA was positively related to the reduction in plasma NEFA concentration for PP ( $P < 0.05$ ). Besides oxidation, the pool of Ac-CoA is decreased by ketogenesis and hydrolysis and export as acetate. Further research is required to determine the fate of Ac-CoA within the timeframe of meals and the effects of feeding on energy charge in hepatic tissue.

**Key Words:** acetyl CoA, metabolic control of intake, postpartum

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**0670 Overconditioned prepartum cows exhibit a greater magnitude of insulin resistance and mobilize more NEFA earlier compared with lean cows.** J. E. Rico\*<sup>1</sup> and J. W. McFadden<sup>1,2</sup>, <sup>1</sup>West Virginia University, Morgantown, <sup>2</sup>Johns Hopkins University, Baltimore, MD.

Overconditioned transition cows are at greater risk of developing metabolic disease compared with lean cows. Severity of metabolic disease can be augmented by the magnitude of insulin resistance. Our objective was to identify the onset of insulin resistance in overconditioned prepartum cows to delineate the progression of the disease for future predictive biomarker discovery. Multiparous Holstein cows were allocated into two treatment groups according to their BCS at d -30 prepartum: lean (LEAN; BCS < 3.25;  $n = 21$ ) or overconditioned (OVER; BCS > 3.75,  $n = 26$ ). Diets were formulated to meet nutrient recommendations. Blood samples were collected at d -45, -30, -15 and -7, relative to expected calving, and at d 1 and 4 postpartum. Plasma glucose, NEFA, insulin, and BHBA concentrations were measured, and the Revised Quantitative Insulin Sensitivity Check Index (RQUICKI) was calculated as an insulin sensitivity indicator. The statistical model included the random effect of cow and the fixed effects of BCS and time (relative to calving). BCS was different for LEAN and OVER at d -30 postpartum ( $3.04 \pm 0.042$  vs.  $3.91 \pm 0.038$ ;  $P < 0.001$ ). With the exception of glucose, plasma variables were affected by time ( $P < 0.001$ ). NEFA (mM) were higher for OVER relative to LEAN at d -45, -30, -15, and -7 (+54%,  $P < 0.01$ ; +40%,  $P < 0.05$ ; +116%,  $P < 0.001$ ; and +91%,  $P < 0.001$ , respectively) and tended to be higher at d 1 (+31%,  $P = 0.07$ ). Insulin ( $\mu\text{U/ml}$ ) was higher in OVER relative to LEAN at d -15 (+37%;  $P < 0.05$ ). RQUICKI was lower for OVER relative to LEAN at d -30, -15, and -7 (-10%,  $P < 0.05$ ; -18%,  $P < 0.001$ ; and -9%,  $P < 0.01$ , respectively), and tended to be lower at d -45 and d 1 for OVER relative to LEAN (-8 and -7%, respectively,  $P = 0.08$ ). BCS affected NEFA and RQUICKI (+44% and -9%, respectively, OVER relative to LEAN;  $P < 0.001$ ). Glucose and BHBA were not affected by BCS. Relative to LEAN, OVER showed a negative change in BCS from d -30 to d -7 (0.13 vs. -0.10 units,  $P < 0.01$ ). BCS had no effects on BW or milk yield. Somatic cell score was higher in OVER at d 10 (+18%,  $P = 0.025$ ). Overconditioned cows experienced a greater magnitude of insulin resistance and mobilized more NEFA earlier compared with lean cows. Early detection of pre-onset insulin resistance in overconditioned dairy cows is needed to develop interventions aimed at reducing excessive NEFA mobilization and associated metabolic disorders.

**Key Words:** insulin resistance, overconditioned, transition cow

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**0671 Identifying biomarkers for pre-onset insulin resistance using mass spectrometry-based metabolomics: Plasma ceramides are elevated in overconditioned transition dairy cows.**

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Metabolomics is a systems biology analytical approach used to study disease phenotypes, an established field in biomedicine that is emerging in the dairy sciences. Parallel with transcriptome and proteome, the comprehensive set of small molecules in biological systems constitutes its metabolome. Because no single analytical methodology is suited to identify all metabolites, a combination of untargeted and targeted methods (measurement of any molecule that ionizes within a specific mass range, and measurement of specific metabolites, respectively) are employed using gas chromatography (GC) or liquid chromatography (LC), coupled with mass spectrometry (MS). Metabolome screening provides the opportunity to discover molecules (biomarkers) that are associated with disease progression. Similar to overweight monogastrics, overconditioned transition dairy cows experience greater insulin resistance compared with lean cows. Since insulin resistance accelerates NEFA mobilization, overconditioned dairy cattle are at greater risk of developing postpartum disease. Therefore, our objective was to screen the bovine metabolome using GC/MS and LC/MS technologies in search for metabolic phenotypes associated with decreased insulin sensitivity. Our data set included multiparous Holstein cows grouped according to BCS at d -30 prepartum: lean (BCS < 3.0;  $n = 10$ ; LEAN) or overconditioned (BCS > 4.0,  $n = 11$ ; OVER), with blood samples collected at d -45, -30, -15 and -7, relative to expected calving, and at d 4 postpartum. For untargeted detection, derivatized plasma methanol extracts were analyzed using GC/MS. For targeted analysis, plasma chloroform-methanol extracts were analyzed by LC/MS. Following normalization, log transformation, and median-scaling, data were analyzed using ANOVA and cluster analysis. Non-parametric spearman's correlations were used to evaluate the associations between NEFA and insulin sensitivity to ceramides. Relative to LEAN, OVER had reduced insulin sensitivity and greater NEFA mobilization at d -30, -15 and -7 prepartum ( $P < 0.05$ ). GC/MS and LC/MS analysis detected lactate, urea, glycerol, amino acids, citrate, mono- and disaccharides, saturated and unsaturated free FA, uric acid, vitamin E, nonesterified cholesterol, ceramides, hexylceramides, and others. Of interest, relative to LEAN, OVER had elevated ceramides at d -15, -7, and 4 (e.g., C20:0-ceramide;  $P < 0.05$ ), and mono- and dihexylceramides at d4 ( $P < 0.05$ ). Multiple ceramides were positively associated with NEFA, and negatively associated with insulin sensitivity (e.g., C22:0-, C24:0-, C24:1-, and C26:0-ceramide;  $P < 0.05$ ). Currently used markers (e.g., NEFA and BHBA) have limited predictive power for pre-onset insulin resistance, as they are

delayed indications of metabolic stress. Metabolomics may improve our ability to predict prepartum cows at risk of developing greater insulin resistance.

**Key Words:** insulin resistance, metabolomics, transition cow

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**0672 Effects of yeast product supplementation on production, feeding behavior, and metabolism in transition dairy cows.** K. Yuan<sup>\*1</sup>, T. Liang<sup>2</sup>, M. Muckey<sup>1</sup>, L. Mendonca<sup>1</sup>, L. Hulbert<sup>1</sup>, L. Mamedova<sup>1</sup>, C. C. Elrod<sup>3</sup>, and B. Bradford<sup>1</sup>, <sup>1</sup>Kansas State University, Manhattan, <sup>2</sup>GM Powertrain, Pontiac, MI, <sup>3</sup>Vi-COR, Inc., Mason City, IA.

The objective of this study was to assess the effects of supplementing a yeast product derived from *Saccharomyces cerevisiae* on production, feeding behavior, and metabolism in transition cows. Forty multiparous Holstein cows were blocked by expected calving date and randomly assigned within block to one of four treatments ( $n = 10$ ) from 21 d before expected calving to 42 d postpartum. Rations were top-dressed with yeast culture plus enzymatically hydrolyzed yeast (Celmanax, Vi-COR, Mason City, IA) at the rate of 0, 30, 60, or 90 g/d throughout the experiment. Dry matter and water intake, feeding behavior, and milk production were monitored. Plasma collected on -21, -7, 1, 4, 7, 14, 21, and 35 d relative to calving was analyzed for glucose,  $\beta$ -hydroxybutyrate, non-esterified fatty acids (NEFA), and haptoglobin. Data were analyzed using mixed models with repeated measures over time. Pre- or postpartum DMI and water intake did not differ ( $P > 0.10$ ) among treatments. There were quadratic dose effects ( $P < 0.05$ ) for prepartum feeding behavior, reflecting decreased meal size, meal length, and intermeal interval, and increased meal frequency for cows received 30 and 60 g/d of yeast products. Postpartum feeding behavior was unaffected ( $P > 0.10$ ) by treatments. Milk yields were not affected ( $P > 0.10$ ; 45.3, 42.6, 47.8, and 46.7 kg/d for 0, 30, 60, and 90 g/d, respectively) by treatments. Tendencies toward increased ( $0.05 < P \leq 0.10$ ) percentages of milk fat, protein, and lactose were detected for cows receiving yeast. A treatment  $\times$  wk interaction ( $P < 0.01$ ) was observed for somatic cell linear score (SCS), reflecting a quadratic dose effect on SCS in wk 1 ( $P = 0.03$ ; 2.34, 2.85, 1.47, and  $4.05 \pm 0.57$  units for 0, 30, 60, and 90 g/d, respectively). Yeast product increased ( $P < 0.01$ ) plasma  $\beta$ -hydroxybutyrate and tended to decrease (quadratic  $P = 0.06$ ) glucose, but did not affect NEFA or haptoglobin. Yeast product supplementation during the transition period did not affect milk production, but may modulate feeding behavior, mammary gland health, and metabolism.

**Key Words:** production and metabolism, transition cow, yeast



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**0673 Milk production performance of autumn-calving Holstein Friesian cows managed under flat-rate or feed-to-yield concentrate feeding systems.**

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This experiment was conducted to examine the effects of two feeding systems, on milk production, body condition score and feed inputs of autumn-calving cows. Autumn-calving Holstein Friesian cows ( $n = 84$ ) were blocked pre-partum according to parity, body condition score, genetic merit for milk production and assigned to one of two postpartum concentrate feeding systems; feed to budget (FTB) or feed to yield (FTY); FTB cows were allocated concentrate at a flat rate, and FTY were allocated concentrate based on individual milk production. Both feeding systems were offered 14.5 kg forage DM/cow/day during the winter housing period containing grass silage and maize silage (in the ratio 2:1) plus 3.0 kg DM of concentrate. Cows on the FTB treatment received a further 3.95 kg DM of concentrate in the milking parlor; cows on the FTY treatment received 0.43 kg DM of concentrate in the milking parlor per kg of milk (based on the previous weeks mean milk production) exceeding a base milk yield 23.0 kg/cow/day. Cows were turned out to pasture on February 10. During the main grazing period (March-September) pre-grazing herbage mass was 1300 to 1500 kg DM/ha (above 4 cm), and was grazed to a residual of < 100kg DM/ha (target 4.0 cm). Where pasture supply was adequate, the FTY group was offered 0.43 kg DM concentrate per kg milk, exceeding a base yield of 25.0 kg/day as described previously. When pasture supply was below the demand, additional concentrate (plus silage if required) was allocated at a flat-rate to both treatments. Data were analyzed using a mixed model procedure in SAS v9.3 which included a repeated measure for week of experiment. Concentrate feeding system had no significant effect on milk yield, milk solids production or mean BCS during the indoor period or at grazing. In total the FTY treatment was fed 250 kg DM/cow less concentrate than the FTB ( $P < 0.001$ ). The ratio of concentrate fed per kg of milk output was lower for FTY which indicates improved efficiency of milk production from forage.

**Key Words:** feed to yield, pasture, autumn calving

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**0674 Does concentrate allocation pattern affect the milk production of autumn calving cows at high and low feeding levels?**

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The objective of this experiment was to examine the effects of concentrate allocation pattern at two concentrate feeding levels, on feed intake, milk yield, and milk composition of autumn calving cows offered a base forage mix of grass silage and maize silage. The study used a randomized block design with a  $2 \times 2$  factorial arrangement of treatments and was performed over an 11-wk period in 2011 and 2012. The treatment factors were flat (F) or variable (V) concentrate allocation pattern, at high (7.0 kg DM/cow per day (Hi)) or low (4.0 kg DM/cow per day (Lo)) concentrate feeding level. Cows ( $n = 108$ ) were blocked in groups of four based on calving date, parity and the milk yield, body weight and BCS of weeks three and four of lactation. Within each treatment cows were sub-grouped as high, medium, and low milk yield based on pre-experimental milk data. The HiV cows received 8.7, 7.0, or 5.3 kg DM of concentrate/cow per day in the high, medium and low subgroups, respectively. The LoV cows received 5.3, 4.0, or 2.7 kg DM of concentrate/cow per day in the high, medium and low subgroups, respectively. Flat rate treatments received 7.0 kg DM (HiF) or 4.0 kg DM (LoF) of concentrate/cow per day. Concentrate was allocated in the base diet; 2 kg and 2.4 kg DM for Hi and Lo, respectively, and the remaining amount was allocated in the parlor. All cows remained on a fixed level of concentrate for the duration of the experiment. Data were analyzed using a mixed model procedure in SAS v9.3, which included a repeated measure for week of experiment. Cows on the Hi treatment had 2.9 kg higher total DM intake ( $P < 0.001$ ), and 0.5 kg reduction in base feed DM intake ( $P < 0.05$ ), compared to animals on the Lo treatment, which had a total DM intake of 15.8 kg DM and a base feed intake of 14.2 kg DM. Each additional 1 kg concentrate DM intake above the Lo treatment, resulted in a 0.43 kg increase in milk yield ( $P < 0.001$ ) which was 25.1 kg and 23.8 kg on Hi and Lo, respectively. There was no allocation pattern by feeding level interactions for milk yield, milk composition or dry matter intake. Allocating concentrate at a variable rate had no effect on milk yield or DM intake, when compared to allocating concentrate at a flat rate.

**Key Words:** feed-to-yield, flat-rate: Holstein Friesian