663 The effect of increasing the nutrient and amino acid concentration of whole milk diets on dairy heifer individual feed intake, growth, development and lactation performance. J. K. Margerison*, *IFNHH Massey University, Palmerston North, New Zealand.*

Whole milk is known to less than an ideal feed for growing animals, while increasing the early (<3 mo.) growth rate of calves has been found to increase the milk production potential of dairy heifers. The aim of this research was to compare the effect of offering whole milk with added carbohydrate and amino acids on the growth, development and weaning age of Holstein Friesian dairy heifers. A total of 60 calves were selected at random and allocated (24 h of age) according to; birth date, breed, stature and live weight to one of 3 treatments; 0 to 18 d all were offered milk and probiotics and at 21 d of age calves were offered either; 4 l/h/d of whole milk (M), 4 l/h/d of whole milk, plus 200 g plant extracts (MP) 200 g plant extracts with amino acids (MPA). All calves weaned at set target weight of 90 kg, when calves were limited to 2 kg/h/d and pasture. Total milk used (l) was; M: 333, MP: 315.4, MPA: 302.1 and plant extract used (kgFM) was; M: 0, MP: 11.6, MPA: 10.9. Pelleted feed intake was significantly lower for calves offered MPA during the milk period (kgFM) (M: 68.3 a, MP: 60.9 a, b, MPA: 56.9 b, (sem 2.57) $P = 0.012$) as was total pellet intake (kgFM) (M: 82.3 a, MP: 74.9 a, b, MPA: 70.9 b, (2.57) $P = 0.012$), while straw intake (kgFM) did not differ (26.8, 24.5, 22.5 (1.4) $P = 0.098$). Calves offered MPA had significantly higher mean weight gain (g/d) (M: 765.5 b, MP: 814.9 a, b, MPA: 876.3 a (25.25) $P = 0.008$) and had a lower number of days to weaning from milk (M: 83.26 a, MP: 78.84 a, b, MPA: 75.53 b (1.88) $P = 0.012$)) and a higher mean hip width gain (mm) (M: 6.1 b, MP: 6.6 a, b, MPA: 7.4 a, (0.34), $P = 0.033$), while mean gain in hip height did not differ (M: 14.0, MP: 15.6, MPA: 15.9 (1.16) $P = 0.476$) compared with calves offered milk. Live weight, maturity levels and hip height and width was not significantly different at parturition, but milk yield and milk fat and protein yield were significantly higher in animals offered additional carbohydrates and AA with whole milk.

Key words: lactation, heifer nutrition, milk production

664 Integration of cyclic GMP-dependent protein kinase (PKG) and phosphatidylinositol 3-kinase (PI3K) on rumen protozoal chemotaxis to glucose and soluble peptides. H. L. Diaz* and J. L. Firkins, *The Ohio State University, Department of Animal Science, Columbus.*

Insulin (Ins) was hypothesized to activate PI3K, increase growth rate, deplete substrate, and increase protozoal chemotaxis. Wortmannin (WORT) is a specific inhibitor of PI3K, and sodium nitroprusside (SNP) activates PKG to polarize cells toward a chemoattractant gradient. We expected chemotaxis for both isotrichids (IS) and entodioniomorphids (EN) toward glucose and for peptides only by EN but hypothesized interactions with the following modifiers at higher dosages. Rumen fluid was collected 3 h after feeding and pre-incubated for 0 and 3 h with Control, 2.5 or 25 μM Ins, 50 or 500 μM SNP and 20 or 200 μM WORT. Two capillary tubes (75 mm) were not filled and uncapped (UN) (control for the protozoal count in beakers) or filled with either saline (Sal; control for random swimming), 1 M glucose (Glc), 1 g/L soluble peptides (Pep), or Glc+Pep and capped before insertion in beakers. In a randomized incomplete block design (blocked for 3 replicate runs), data were analyzed by protected LSD. For EN, the log10 counts in capillary tubes (20 minus 0 min) differed ($P < 0.05$) for UN by pre-incubation treatment, so data were covariate-adjusted to the mean UN (3.54 log10 counts). Main effects means were 2.31, 2.91, 2.81, and 3.22 for SAL, Glc, Pep, and Glc+Pep, respectively. Glc+Pep were additive at increasing chemotaxis, regardless of SNP or WORT. The interaction ($P < 0.05$) between pre-incubation beaker treatments and capillary chemoattractant treatments was from log10 counts being 2.41, 2.48, 2.28, 2.37, 2.05, 2.52, and 2.06 for Sal but 2.70, 2.86, 2.70, 2.92, 3.04, 2.92, and 3.22 for Glc for CONTROL, Ins2.5, Ins25, SNP50, SNP500, WORT20, and WORT200, respectively. For Sal, the SNP500 and WORT200 counts were decreased ($P < 0.05$) vs. CONTROL, but Glc increased ($P < 0.05$) chemotaxis for SNP500 and WORT200 vs. CONTROL. For IS, main effects means (log10, no pre-incubation treatment effect) were 1.56, 2.33, 1.11, 1.62, and 1.96 for Sal, Glc, Pep, Glc+Pep, and UN; Pep decreased ($P < 0.05$) chemotaxis. These data justify future experiments to integrate chemotaxis and growth rate.

Key words: rumen protozoa, chemotaxis, glucose

665 Evaluation of specificity of hydrolysis methods for separation of water-soluble carbohydrates. M. B. Hall*, *US Dairy Forage Research Center, USDA-ARS, Madison, WI.*

Various hydrolysis methods have been recommended to convert oligo- or polymeric water soluble carbohydrates to monomers for detection in reducing sugar assays, but responses and specificity for different carbohydrates have not been well characterized. The study objective was to evaluate reducing sugar responses of sucrose (SUC), maltose (MAL), lactose (LAC), raffinose (RAF), and inulin (INU) to hydrolysis (HYD) by invertase, 0.5 M HCl, or 0.037 M H_2SO_4 or a mixture of sucrase/maltase/pullulanase/β-amylase (Sucrase Mix). The p-hydroxybenzoic acid hydrazide reducing sugar method was used for detection. Hydrolyzed and unhydrolyzed samples were analyzed in 2 runs for each method. The statistical model included carbohydrate (WSC), HYD method, and the interaction (INT). Values presented are least squares means for reducing sugars as a percentage of sample DM. Unhydrolyzed carbohydrates differed in reducing sugar values ($P < 0.01$; SUC = 1.1%, MAL = 54%, LAC = 42%, RAF = 2.0%, and INU = 7.4%, SED = 1.5%). LAC and MAL values reflect reactivity of the reducing end hexose in both molecules, whereas RAF and SUC lack reactive reducing end hexoses. The value for INU may reflect presence of a small amount of damaged molecules. Hydrolyzed minus unhydrolyzed values describe the amount of reducing sugar released by hydrolysis. This difference differed by WSC ($P < 0.01$), HYD ($P < 0.01$), and INT ($P < 0.01$) (Table 1; SED = 3.5%). LAC was largely unaffected by HYD. Because of complete or partial hydrolysis of SUC, RAF, and INU by the same treatments, and responses of unhydrolyzed LAC and MAL, it is concluded that these hydrolyses do not allow separation of specific WSC.

Table 1. Hydrolyzed minus unhydrolyzed reducing sugar values by hydrolysis method, % of DM

	Hydrolysis Method			
Carbohydrate	Sucrase Mix	Invertase	HCl	H_2SO_4
Sucrose	84	103	101	102
Maltose	35	2.2	4.6	2.2
Lactose	0.6	3.1	2.8	1.4
Raffinose	0.4	91	89	92
Inulin	4.0	19	97	99

Key words: sugars, method

666 Effect of dietary protein level and rumen-protected amino acid supplementation on dietary amino acid apparent digestibility and recovery in milk in lactating dairy cows. C. Lee*[1], A. N. Hristov[1], T. Cassidy[1], K. Heyler[1], H. Lapierre[2], G. A. Varga[1], and C. Parys[3], *[1]Pennsylvania State University, University Park, [2]Agriculture and Agri–Food Canada, Sherbrooke, QC, Canada, [3]Evonik Degussa GmbH, Hanau, Germany.*

This study investigated the effect of dietary CP level and rumen-protected Lys and Met (RPLys and RPMet) supplementation on apparent total tract digestibility (ATTD) of amino acids (AA) and recovery in milk protein in dairy cows. The experiment was conducted with 8 Holstein cows (102 ± 28 DIM; 26.0 ± 0.79 kg/d DMI; 40.9 ± 1.46 kg/d milk yield) in a replicated 4 × 4 Latin square design trial with 21–d periods. Treatments were: 15.6% CP diet [HighCP; metabolizable protein (MP) balance: –24 g/d], 14.0% CP diet (LowCP, MP balance: –283 g/d), LowCP supplemented with RPLys (AminoShure–L, estimated 24 g/d digestible Lys supply; LowCPLys), and LowCP diet supplemented with RPLys plus RPMet (Mepron®, estimated 15 g/d digestible Met supply; LowCPLysMet). Data were analyzed using the mixed procedure of SAS with diet, square and period in the model and animal within group as a random effect. Plasma Met concentration was increased (30%; $P = 0.02$) by LowCPLysMet compared with LowCP. RPLys supplementation had no effect on plasma Lys. LowCPLys and LowCPLysMet increased ($P < 0.001$) Lys ATTD compared with HighCP and LowCP (51, 47, and 39%, respectively). Met ATTD was higher ($P < 0.001$) for LowCPLysMet compared with the other diets (54 vs. average of 44%). His, essential AA (EAA), and total AA ATTD were higher ($P = 0.001$ to 0.04) for HighCP compared with the LowCP diets. All AA secretion in milk was not affected by diet. RPLys and RPMet supplementation decreased ($P < 0.001$) milk Lys (by 25%) and Met (by 30%, respectively) recoveries (milk ÷ intake) compared with LowCP. Lys, His, EAA and total AA recoveries in milk were greater ($P < 0.001$ to 0.07) for the LowCP diets compared with HighCP. In conclusion, supplementation of LowCP with RPLys and RPMet increased ATTD of total intake Lys and Met, respectively. Supplementation, however, reduced the apparent efficiency of utilization of total intake Lys and Met for milk protein secretion. The apparent efficiency of utilization of all dietary AA for milk protein secretion was increased by decreasing dietary protein intake.

Key words: dietary protein, amino acid utilization, dairy cow

667 Microbiome analysis of the rumen, cecum, and feces of dairy cows with subacute ruminal acidosis. E. Khafipour[1], S. Li*[1], J. C. Plaizier[1], S. E. Dowd[2], and D. O. Krause[1], *[1]University of Manitoba, Winnipeg, MB, Canada, [2]Medical Biofilm Research Institute, Lubbock, TX.*

Subacute ruminal acidosis (SARA) is a metabolic disease common to high producing dairy cattle that is characterized by daily episodes of low rumen pH. It has been assumed that SARA primarily afflicts the rumen, but it may also affect the large intestine. The effects of SARA on the rumen, cecum and fecal microbiome were determined using next-generation sequencing. Six nonlactating Holstein cows with cannula in the rumen and the cecum were used in a replicated 3 × 3 Latin square. During the first 3 wk of each 4 wk experimental period, cows received a control diet containing 70% forage (DM basis). During wk 4 of each period, cows received one of the 3 diets; a control diet, a grain-based SARA challenge (GBSC) diet containing 64% concentrate including 34% wheat-barley pellets, or an alfalfa-pellet SARA challenge (APSC) diet containing 56% forage of which 37% was alfalfa pellets. Rumen, cecum and fecal samples (n = 54) were collected at 6h after feed delivery in wk 4. DNA was extracted from the samples and subjected to pyrosequencing of 16S rRNA. Sequence coverage was assessed with rarefaction and was 74.8%, 81.0%, and 77.7% for the rumen, cecum and fecal samples, respectively. In all compartments, the number of operational taxonomic units was highest in controls followed by APSC and GBSC ($P < 0.05$). Species richness and diversity was lowest in GBSC ($P < 0.05$). A total of 10 phyla were represented in all data sets. If a phylum was present in all animals at 1% or higher, it was considered a core-phylum. Bacteroidetes and Firmicutes were core phyla in all compartments. Proteobacteria and Spirochaetes only core to the rumen, and Fusobacteria only core in the cecum. The Bacteroidetes population was most abundant in the rumen (40% compared with 25% in the cecum and 17% in the feces) while Firmicutes were lowest (53% compare with 65% in the cecum and 80% in the feces). The increases in Rikenella, Butyrivibrio, and Treponema in the rumen were associated with APSC. The loss of Fusobacteria in the cecum and Lentisphaerae in the feces was associated with GPSC ($P < 0.05$). The cecum and fecal microbiome of GBSC was clearly distinct from other groups.

Key words: SARA, gastrointestinal tract, microbiome

668 The effect of diet on milk fatty-acid profiles in Holstein dairy cattle on commercial dairy farms. R. W. Swidan*[1], Y. Chouinard[2], R. Lacroix[1,3], D. Lefebvre[3], and K. M. Wade[1], *[1]McGill University, Montreal, QC, Canada, [2]Laval University, Quebec City, QC, Canada, [3]Valacta, Ste. Anne de Bellevue, QC, Canada.*

Milk samples were collected from 14 commercial dairy farms in Québec with a view to investigating the effects of diet on fatty-acid (FA) profiles. Data represented 284 individual cows covering the period 2006 to 2008. Feed information was obtained from the Québec DHIA (Valacta). The objective of the study was to determine the effects of main forage source (corn silage, grass silage, legume silage, and hay), main energy source (corn, barley, both corn and barley, mixed meal and "other concentrates"), and the presence or absence of both corn silage and corn grain, on the FA profile of Holstein milk. More specifically, the 3 groups investigated were omega-3 FA, conjugated linoleic acids (CLA), and trans FA. Samples were analyzed in a repeated-measure model using the PROC MIXED (SAS). Farms feeding grass silage and corn silage as a main source of forage were found to have lower concentrations ($P < 0.001$) of total omega-3 FA (0.59 and 0.63 g/100g FA, respectively) in the milk versus farms feeding legume silage or hay (0.85 and 1.11 g/100g FA, respectively), while farms feeding hay produced milk with more CLA (0.77 g/100 g FA) compared with those fed grass silage (0.44 g/100g FA). The presence of any corn silage in the diet versus no corn silage resulted in a significant decrease ($P < 0.005$) in total omega-3 FA (0.75 vs. 0.91 g/100g FA, respectively).

Corn silage also tended to decrease both CLA and trans FA concentration, but neither was significant ($P > 0.01$). Different sources of energy in the diet had no significant effect on total omega-3 FA in the milk fat. With regard to CLA, mixed-meal based diets led to a significant increase ($P < 0.001$) in the concentration, while the presence of any corn yielded significantly lower concentrations ($P < 0.001$) than all other energy sources. "Other concentrates" were intermediate in their effect on CLA. The results suggest that changing the diet of dairy cows on commercial farms may affect the fatty acid profile of milk fat, which may have an impact on its processing quality or nutritive value.

Key words: concentrates, forages, milk fatty acids

669 Effects of close-up dietary energy strategy and prepartal dietary monensin on production and metabolism in Holstein cows. J. A. Vasquez*[1], K. L. Perfield[2], H. B. Green[2], and J. K. Drackley[1], *[1]University of Illinois, Urbana, [2]Elanco Animal Health, Greenfield, IN.*

Effects of higher-energy close-up (CU) diets compared with single-group controlled energy (CE) diets remain controversial. Benefits of prepartal monensin (M) in each dietary strategy have not been determined. In a randomized design, multiparous (n = 70) and primiparous (n = 32) cows were assigned to 1 of 4 treatments in a 2 (dry period diet) x 2 (monensin supplementation) factorial arrangement. Dry period diets were either a single CE diet (1.30 Mcal NEL/kg DM) or CE in the far-off period followed by 21 d of CU (1.49 Mcal NEL/kg DM). Diets were either unsupplemented or supplemented with M (22 g/T). Cows received diets for a target of 50 d prepartum. After parturition all cows received a common lactation diet (1.70 Mcal NEL/kg DM) that contained M (14 g/T); data were collected for 84 d. Dry period diet did not affect DMI during the far-off period ($P = 0.21$), but DMI was greater ($P < 0.001$) during the close-up period for cows fed CU; DMI was unaffected by M in either period. Neither diet nor M affected DMI, BW, or BCS postpartum. Prepartum diet did not affect milk yield, milk protein percent, yields of milk components, or FCM but milk fat percent was lower ($P = 0.03$) for CE than for CU. Prepartal M tended ($P = 0.06$) to increase milk yield and increased ($P < 0.01$) lactose content in milk. Contents of fat, protein, and total solids were not affected by M, but yields of fat ($P = 0.01$), lactose ($P = 0.03$), and total solids ($P = 0.03$) as well as FCM ($P = 0.01$) were greater for cows fed M prepartum. Prepartal NEFA were greater ($P < 0.01$) but postpartum NEFA were lower ($P = 0.05$) for cows fed CE. Concentrations of NEFA and BHBA were not affected pre- or postpartum by prepartal M. Concentrations of total lipid, triacylglycerol, and glycogen in a subset of mature cows at d −10 and d 7 were unaffected by diet or M. Other than increasing milk fat content, probably due to greater NEFA, feeding a higher-energy CU diet did not benefit production or metabolism. Prepartum supplementation of M increased yields of milk fat, lactose, total solids, and FCM.

Key words: monensin, prepartum dietary energy, metabolism

670 Effects of close-up dietary energy strategy and prepartal dietary monensin on rumen dynamics and fermentation in Holstein cows. B. F. Richards*[1], J. A. Vasquez[1], K. L. Perfield[2], H. B. Green[2], M. R. Murphy[1], and J. K. Drackley[1], *[1]University of Illinois, Urbana, [2]Elanco Animal Health, Greenfield, IN.*

Concern exists about postpartal rumen adaptation in cows fed single-group controlled energy (CE) diets prepartum rather than traditional close-up (CU) diets. Effects of prepartal monensin (M) on rumen adaptation with these diets are unknown. Multiparous cows (n = 16) with ruminal cannulas were assigned to treatments in a 2 (diet) x 2 (M supplementation) factorial arrangement. Prepartum diets fed for 50 d were either a single CE diet (1.30 Mcal NEL/kg DM) or CE in the far-off period followed by 21 d of CU (1.49 Mcal NEL/kg DM). Each diet was fed without and with M (24.2 g/tonne). Postpartum all cows received a common lactation diet (1.70 Mcal NEL/kg DM) containing M (15.4 g/tonne) for 84 d. Rumen measurements were made at d −14, 2, 14, and 28 relative to parturition. Neither diet nor M affected DMI or BW pre- or postpartum. Supplemental M increased ($P < 0.01$) milk yield. Mass of rumen contents decreased ($P < 0.05$) from d −14 (59.4 kg) to d 2 (45.9 kg) but did not differ among treatments. Rumen fluid dilution rate decreased when M was added to CE but increased when M was added to CU (diet x M interaction, $P < 0.01$). Rumen particulate passage rate did not differ among diets or days. Ruminal pH was higher ($P < 0.01$) at d −14 (6.58) than postpartum (6.23, 6.30, 6.23 for d 2, 14, 28) but did not differ by diet or M. Total VFA concentration tended to increase when M was fed with CE but decreased slightly when M was fed with CU (diet x M, $P = 0.09$). Total VFA decreased at d 2 for cows not fed M (96.9 mM) but increased at d 2 (132.1 mM) when M was fed (M x day, $P < 0.01$). Acetate (% of total VFA) tended to decrease when M was fed with CE but increased when M was added to CU (diet x M, $P = 0.10$); propionate followed the opposite trend ($P = 0.10$). Butyrate tended ($P = 0.09$) to be greater for CE. Mean papillae length was decreased by M at d 2 mainly in cows fed CE (diet x M x day, $P = 0.02$). Feeding a CU diet had few effects on rumen characteristics compared with the single CE diet. Supplemental M shifted rumen fermentation differently between diets and modulated changes in rumen environment across the transition.

Key words: monensin, prepartum dietary energy, rumen